(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 July 2004 (29.07.2004)

PCT

(10) International Publication Number
WO 2004/062591 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/US2004/000320

(22) International Filing Date: 6 January 2004 (06.01.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/438,343 6 January 2003 (06.01.2003) US
60/473,246 22 May 2003 (22.05.2003) US

(71) Applicant *(for all designated States except US)*: **OREGON HEALTH AND SCIENCE UNIVERSITY** [US/US]; 2525 Sw First Avenue, Suite 120, Mail Code Ad120, Portland, Or 97201 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **MOSES, Ashlee** [US/US]; 2525 Sw First Avenue, Suite 120, Mail Code Ad120, Portland, Or 97201 (US). **FRUH, Klaus** [US/US]; 2525 Sw First Avenue, Suite 120, Mail Code Ad120, Portland, Or 97201 (US). **KING, Jeffrey, S.** [US/US]; 2525 Sw First Avenue, Suite 120, Mail Code Ad120, Portland, Or 97201 (US). **HICKS, James, B.** [US/US]; 2525 Sw First Avenue, Suite 120, Mail Code Ad120, Portland, Or 97201 (US). **RAGGO, Camilo** [US/US]; 2525 Sw First Avenue, Suite 120, Mail Code Ad120, Portland, Or 97201 (US). **NELSON, Jay** [US/US]; 2525 Sw First Avenue, Suite 120, Mail Code Ad120, Portland, Or 97201 (US).

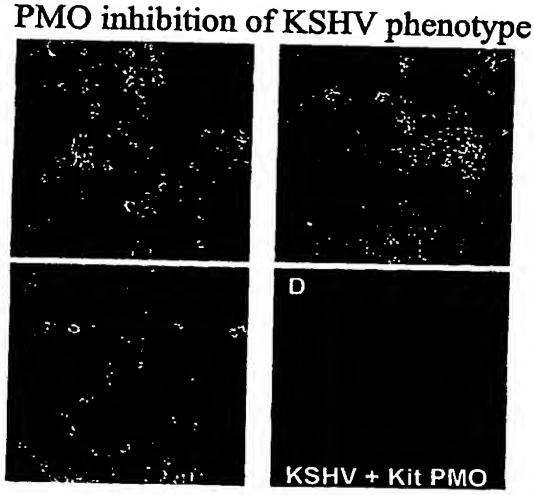
(74) Agents: **DAVISON, Barry, L.** et al.; 2600 Century Square, 1501 Fourth Avenue, Seattle, WA 98101-1688 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

[Continued on next page]

(54) Title: METHODS OF TREATMENT AND DIAGNOSIS OF KAPOSI'S SARCOMA (KS) AND KS RELATED DISEASES



(57) Abstract: The present invention uses gene expression profiling, and gene silencing methods to identify and provide a plurality of 'validated' KSHV-induced cellular gene sequences and pathways useful as targets for modulation of KSHV-mediated effects on cellular proliferation and phenotype (e.g., cancer) associated with latent and lytic phases of the Kaposi's sarcoma-associated herpesvirus (KSHV; Human herpesvirus 8; HHV8) life cycle. Particular embodiments provide therapeutic compositions, and methods for modulation of KSHV infection or KSHV-mediated effects on cellular proliferation and phenotype, comprising inhibition of KSHV-induced gene sequences. Additional embodiments provide screening assays for compounds useful to modulate KSHV infection or KSHV-mediated effects on cellular proliferation and phenotype. Further embodiments provide diagnostic and/or prognostic assays for KSHV infection.

WO 2004/062591 A2

GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

METHODS OF TREATMENT AND DIAGNOSIS OF KAPOSI'S SARCOMA (KS) AND KS RELATED DISEASES

FIELD OF THE INVENTION

The present invention relates to the identification and use of modulators of KSHV-induced cellular gene expression. Preferred modulators are inhibitors capable of reducing the expression of KSHV-induced genes, reducing or preventing the expression of mRNA from KSHV-induced genes, or reducing the biological activity of corresponding KSHV-induced cellular gene products. The invention provides therapeutic methods, diagnostic methods and compositions useful for the treatment of Kaposi's sarcoma (KS) and related cancers. Particular embodiments also provide drug candidate screening assays. The present invention uses nucleic acid microarrays and gene expression profiling, along with antisense oligonucleotide methods to identify and validate, respectively, therapeutically useful gene targets that are regulated upon KSHV infection of endothelial cells.

BACKGROUND

Kaposi's Sarcoma (KS) is the most frequent malignancy afflicting AIDS patients. KSHV (or human herpesvirus 8, HHV8) is consistently associated with all epidemiologic forms of KS and is recognized as the etiologic agent of the disease. KSHV infects the *spindle-shaped cells* that characterize the tumor as well as the corresponding lesional endothelial cell precursors, and infiltrating leukocytes. The tumor lesion is characterized by abnormal vascularization and extensive extravasation of inflammatory cells and erythrocytes. The majority of cells harbor the KSHV genome in a latent form, with a small percentage entering a lytic cycle to produce infectious virus.

Various KSHV genes are known to be capable of deregulating cellular growth, and some of these bear homology to human oncogenes, growth factors, etc., while others are unique (*see e.g.*, Moses et al., *J. Virol.* 76:8383-8399, 2002). Nonetheless, relatively little is known about the influence of viral gene expression on specific cellular gene profiles, or about how such virus-cell interactions contribute to tumorigenesis. Viral gene expression patterns appear to be tumor or stage specific.

Spindle cell formation can be replicated *in vitro* by infection of permissive, human dermal microvascular endothelial cells (DMVEC) with KSHV (Moses et al., *J. Virol.* 73:6892-6902, 1999). Infection of DMVEC with KSHV results in phenotypic alteration, including spindle cell formation, loss of contact inhibition and angiogenesis in soft agar. Thus, KSHV-

DMVEC interactions provide an excellent *in vitro* model system for KS lesion formation *in vivo*, and provide a means to identify those cellular gene sequences regulated in response to KSHV infection.

However, additional methods and studies are needed to distinguish, from among those KSHV-regulated cellular gene sequences, those actually required for KSHV-induced proliferative and phenotypic/developmental changes and which could therefore provide validated intervention targets for the inhibition of KSHV-induced cellular phenomena and the treatment of KSHV-induced hyperproliferative disorders such as cancer. There is a need in the art for such validated targets, and for compositions and methods to affect them.

SUMMARY OF THE INVENTION

Nucleic acid microarray techniques were used in combination with KSHV-infected dermal microvascular endothelial cells (DMVEC) to identify and 'validate' cellular genes and pathways useful in modulating latent and lytic phases of the life cycle of Kaposi's sarcoma-associated herpesvirus (KSHV; Human herpesvirus 8; HHV8). The present Examples show for the first time that modulators of the expression of particular validated KSHV-induced cellular gene targets are suitable agents for treating KSHV-related cancer and hyperplastic/neoplastic conditions.

The present invention provides modulators of KSHV-induced gene expression including, but are not limited to antisense molecules, ribozymes, antibodies or antibody fragments, proteins or polypeptides as well as small molecules. The inventive modulators are useful for reducing the expression of KSHV-induced genes, reducing or preventing the expression of mRNA from KSHV-induced genes, or reducing the biological activity of corresponding KSHV-induced cellular gene products. Preferably, the inventive modulators are directed to one or more validated KSHV-induced gene targets, the expression of which is required, at least to some extent, for KSHV-mediated effects on cellular proliferation and phenotype.

Particular embodiments of the present invention provide therapeutic methods and compositions for modulation of KSHV infection comprising use of inventive modulators for inhibition of the expression of KSHV-induced genes, reducing or preventing the expression of mRNA from KSHV-induced genes, or reducing the biological activity of corresponding KSHV-induced cellular gene products.

Preferred inventive modulators are oligonucleotides, such as antisense molecules, siRNA, or ribozymes, to target and modulate the expression of polynucleotides (*e.g.*, mRNA) comprising KSHV-induced gene sequences.

Preferred antisense molecules or the complements thereof comprise at least 10, 15, 20 or 25 consecutive complementary nucleotides of, or hybridize under stringent or highly stringent conditions to at least one of the nucleic acid sequences from the group consisting of SEQ ID NO:1 (cDNA for RDC1; GPCR RDC1), SEQ ID NO:3 (cDNA for IGFBP-2; insulin-like growth factor binding protein 2), SEQ ID NO:5 (cDNA for FLJ14103 protein), SEQ ID NO:7 (cDNA for KIAA0367 protein), SEQ ID NO:9 (cDNA for Neuritin), SEQ ID NO:11 (cDNA for INSR; insulin receptor), SEQ ID NO:13 (cDNA for KIT; c-kit), SEQ ID NO:25 (LOX cDNA for lysyl oxidase preprotein); SEQ ID NO:27 (NOV cDNA for nov precursor), and SEQ ID NO:29 (ANGPTL2 cDNA for angiopoietin-like 2 precursor). Preferably, such antisense molecules are PMO (phosphorodiamidate morpholino Oligomers) antisense molecules.

Preferred compositions comprise one or more of such modulators or preferred modulators, along with a pharmaceutically acceptable carrier or diluent.

Additional embodiments provide screening assays for compounds useful to modulate KSHV infection.

Further embodiments provide diagnostic or prognostic assays for KSHV infection.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows dermal microvascular endothelial cells (DMVECs) that are uninfected ("Mock") (left-most panel), 1-week post-infection (central panel), or 4-weeks post- infection (right-most panel). The beginning of characteristic *spindle cell* formation in DMVEC cells can be seen 1-week post-infection with KSHV, and substantially progresses through 4 weeks post-infection.

Figure 1B shows red fluorescent staining of latent KSHV infected DMVEC cells ("ORF7," left-most panel), green fluorescent staining of lytic KSHV-infected DMVEC cells ("B-ORF59," central panel), and green fluorescent staining of lytic KSHV-infected DMVEC cells enhanced with PMA ("ORF59+PMA," right-most panel).

Figure 1C shows the beginning of foci formation in KSHV-infected DMVEC at 1-week post infection ("KSHV 1 week," left-most panel), progression of foci formation at 4-weeks post infection ("KSHV 4 weeks," central panel), and KSHV-infected DMVECs growing in soft agar as a result of the acquisition of anchorage-independent growth ("KSHV Agar," right-most panel).

Figure 2 shows a pie-type chart for functional group assignment (described under "EXAMPLE 2" below, based on art-available information) of genes having altered expression in DMVEC in response to KSHV infection.

Figure 3A shows that treatment with c-Kit PMO antisense (SEQ ID NO:21) resulted in restoring contact-inhibited growth of KSHV-infected DMVECs. Specifically, Figure 3A (upper-left panel "A") shows that during the week of post-loading culture, Untreated and control EPEI-treated KSHV-infected DMVECs exhibited loss of contact inhibition, and displayed the capacity to grow in disorganized, multi-layered foci that were evident by day 6 post-loading (upper-left panels "A" and "B," respectively). By contrast, KSHV-infected DMVECs loaded with c-Kit-specific antisense PMO oligonucleotides (+EPEI) did not develop foci, and maintained a quiescent contact-inhibited monolayer (lower-left panel "C").

Figure 3B shows evidence that despite expression in some cells of c-kit protein (red fluorescent staining), the cell cultures treated (loaded) with c-Kit antisense PMO oligomer (SEQ ID NO:) (green fluorescent staining) did not progress to spindle cell and foci formation (*e.g.*, *see* phase contrast images of Figure 3A, lower-left panel "C").

Figures 4A, 4B, 4C and 4D show representative fields of KSHV-infected DMVEC treated with various gene-specific PMO antisense oligonucleotides as indicated, and visualized by CD31 staining: 100% proliferation control (no PMO oligonucleotides) (Figure 4A); RDC-1-specific PMO antisense oligonucleotides, resulting in 43% growth inhibition and full phenotypic inhibition (Figure 4B); KIAA0367-specific PMO antisense oligonucleotides, resulting in 28% growth inhibition and intermediate phenotypic inhibition (Figure 4C); and MFAP-specific PMO antisense oligonucleotides, resulting in 11% growth inhibition and no phenotypic inhibition (Figure 4D). According to the present invention, the extent of PMO-mediated inhibition of KSHV-induced proliferation (% growth inhibition) correlates with the corresponding phenotype inhibition values (full, intermediate and no inhibition).

DETAILED DESCRIPTION OF THE INVENTION

IDENTIFICATION OF KSHV-REGULATED GENES AND PATHWAYS, VALIDATION OF SAME AS THERAPEUTIC TARGETS, AND PROVISION OF THERAPEUTIC MODULATORS

Overview

The present invention uses gene expression profiling, and gene silencing methods to identify and provide a plurality of 'validated' KSHV-induced cellular gene sequences and pathways useful as targets for modulation of KSHV-mediated effects on cellular proliferation and phenotype (*e.g.*, cancer) associated with latent and lytic phases of the Kaposi's sarcoma-associated herpesvirus (KSHV; Human herpesvirus 8; HHV8) life cycle. Validated gene targets correspond to those KSHV-induced gene sequences the expression of which is required, at least

to some extent, for KSHV-mediated effects on cellular proliferation and phenotype. Inventive modulators of validated targets are agents that act by inhibiting the expression of validated KSHV-induced genes, by reducing or preventing the expression of mRNA from validated KSHV-induced genes, or by reducing the biological activity of corresponding KSHV-induced cellular gene products. Inventive modulators of KSHV-induced gene expression include, but are not limited to antisense molecules, siRNA agents, ribozymes, antibodies or antibody fragments, proteins or polypeptides as well as small molecules.

DEFINITIONS

The term "siRNA" or "RNAi" refers to small interfering RNA as is known in the art (*see e.g.*: U.S. Patent 6,506,559; Milhavet et al., *Pharmacological Reviews* 55:629-648, 2003; and Gitlin et al., *J. Virol.* 77:7159-7165, 2003; incorporated herein by reference).

The term "DMVEC" refers to human dermal microvascular endothelial cells.

Soft agar model system for in vivo KSHV-related cancer. Inventive KSHV-related therapeutic targets were identified by the use of a soft agar-based primary dermal microvascular endothelial cell (DMVEC) growth and differentiation assay system, which is an art-recognized model system for cancer *in vivo* (*e.g.*, Tomkowicz, K et al., *DNA Cell Biol.* 21:151, 2002 (use of soft agar assays system to demonstrate transformation with KSHV kaposin protein); Saucier et al., *Oncogene* 21:1800, 2002 (use of soft agar assays system to demonstrate transformation with Met RTK protein); and *see also* Chernicky, CL, *Mol. Pathol.* 55:102, 2002 (use of inhibition of colony formation in soft agar as validation for siRNS inhibition of a tumor growth factor); and EXAMPLE 1 below). In the soft agar system, KSHV-infected DMEC display various hallmarks of KSHV-related *in vivo* cancer, including, but not limited to anchorage-independent growth and spindle cell formation. Significantly, inventive modulators were shown to either inhibit or cause reversion of cancer phenotype (*e.g.*, inhibits formation of spindle cells, or causes reversion of the spindle cells phenotype), and/or to inhibit anchorage-independent growth (EXAMPLES 2 and 3, below).

Identification of KSHV-induced cellular genes using microarrays. Cellular genes involved in the transformed phenotype caused by latent infection with KSHV were identified by using DNA microarrays to examine the differential gene expression profiles of primary dermal microvascular endothelial cells (DMVEC) before and after KSHV-infection. Such microarray technology is well known in the art (*see, e.g.*, Moses et al., *J. Virol.* 76:8383-8399, 2002; WO 02/10339 A2, published 07 February 2002; Salunga et al., *In* M. Schena (ed.), DNA

microarrays, A practical approach; Oxford Press, Oxford, United Kingdom, 1999; *and see* Simmen et al., *Proc. Natl. Acad. Sci. USA* 98:7140-7145, 2001; all of which are incorporated by reference herein in their entirety), and can be performed using commercially available arrays (*e.g.*, Affymetrix U133A, U133B and U95A GeneChip® arrays) (Affymetrix, Santa Clara, CA). The Human Genome U133 (HG-U133) set, consists of two GeneChip® arrays, and contains almost 45,000 probe sets representing more than 39,000 transcripts derived from approximately 33,000 well-substantiated human genes (Affymetrix technical information). The set design uses sequences selected from GenBank®, dbEST, and RefSeq (*Id*).

Specifically, as described in detail under EXAMPLE 2 herein, nucleic acid microarray technology was used for gene expression profiling of KSHV-infected DMVEC, relative to non-infected control cells, to identify cellular genes whose expression is regulated by KSHV. Each of the DMVEC infected/uninfected sample comparisons resulted in approximately 480 probe sets with increased expression, with 316 probe sets that showed increased expression in duplicate infections. There were 390 probes sets that showed decreased expression in duplicate, out of approximately 600 probe sets that were down in individual experiments (EXAMPLE 2). The 706 probes sets identified with significant changes in expression correspond to 580 unique gene sequences.

Validation of therapeutic targets by gene silencing using gene-specific PMO antisense compounds. Additionally, particular KSHV-regulated or KSHV-induced gene sequences were identified as *validated* therapeutic targets by specific gene silencing using PMO (phosphorodiamidate morpholino Oligomers) antisense oligonucleotide inhibition in combination with measuring the effects of such gene silencing using cellular differentiation (EXAMPLE 3 below, at TABLE 2) or cellular proliferation assays (EXAMPLE 3 below, at TABLE 4). Silencing of such genes precluded progression into the KSHV-transformed phenotype when silencing occurred prior to transformation, or induced reversion to the normal state when silencing occurred after induction of the transformed state (EXAMPLE 3 below, at TABLE 2).

Therapeutic utility. According to the present invention, PMO-mediated gene silencing using the soft agar growth/differentiation system not only provides validation of therapeutically-significant targets, but also provides gene-specific modulators of KSHV-induced cellular gene expression that have therapeutic utility. PMOs (*see, e.g.*, Summerton, et al., *Antisense Nucleic Acid Drug Dev.* 7:63-70, 1997; and Summerton & Weller, *Antisense Nucleic Acid Drug Dev.* 7:187-95, 1997) represent a class of art-recognized antisense drugs for treating various diseases, including cancer. For example, Arora et al. (*J. Pharmaceutical Sciences* 91:1009-1018, 2002)

demonstrated that oral administration of *c-myc*-specific and CYP3A2-specific PMOs inhibited *c-myc* and CYP3A2 gene expression, respectively, in rat liver by an antisense mechanism of action. Likewise, Devi G.R. (*Current Opinion in Molecular Therapeutics* 4:138-148, 2002) discusses treatment of prostate cancer with various PMO therapeutic agents).

Likewise, siRNA" or "RNAi" agents are emerging as a new class of art-recognized drugs (*see e.g.*: U.S. Patent 6,506,559; Milhavet et al., *Pharmacological Reviews* 55:629-648, 2003; and Gitlin et al., *J. Virol.* 77:7159-7165, 2003; incorporated herein by reference).

Accordingly, the present invention provides therapeutic compositions, and methods for modulation of KSH infection, comprising inhibition of KSHV-induced gene expression (*e.g.*, inhibition of the expression of validated KSHV-induced genes, reducing or preventing the expression of mRNA from validated KSHV-induced genes, or reducing the biological activity of corresponding KSHV-induced cellular gene products).

Additional embodiments provide screening assays for compounds useful to modulate KSHV infection.

Further embodiments provide diagnostic or prognostic assays for KSHV infection.

Preferred Inventive Modulators, Compositions, Utilities and Expression Vectors

Modulators of KSHV-induced gene expression. Particular embodiments provide modulators of KSHV-induced cellular gene expression. Preferably, inventive modulators are directed to one or more validated KSHV-induced cellular gene targets, the expression of which is required, at least to some extent, for KSHV-mediated effects on cellular proliferation and phenotype.

Inventive modulators include, but are not limited to, antisense molecules, ribozymes, antibodies or antibody fragments, proteins or polypeptides as well as small molecules. Particular KSHV-induced gene expression modulators, such as gene-specific antisense and ribozyme molecules, and antibodies and epitope-binding fragments thereof, are *inhibitors* of KSHV-induced gene expression, or of the biological activity of proteins encoded thereby.

Preferably, inventive antisense molecules are oligonucleotides of about 10 to 35 nucleotides in length that are targeted to a nucleic acid molecule corresponding to a KSHV-induced gene sequence, wherein the antisense molecule inhibits the expression of at least one KSHV-induced gene sequence. Antisense compounds useful to practice the invention include oligonucleotides containing art-recognized modifed backbones or non-natural internucleoside linkages, modified sugar moieties, or modified nucleobases.

Preferred antisense molecules or the complements thereof comprise at least 10, at least 15, at least 20 or at least 25, and preferably less than about 35 consecutive complementary nucleotides of, or hybridize under stringent or highly stringent conditions to at least one of the nucleic acid sequences from the group consisting of SEQ ID NO:1 (cDNA for RDC1; GPCR RDC1), SEQ ID NO:3 (cDNA for IGFBP-2; insulin-like growth factor binding protein 2), SEQ ID NO:5 (cDNA for FLJ14103 protein), SEQ ID NO:7 (cDNA for KIAA0367 protein), SEQ ID NO:9 (cDNA for Neuritin), SEQ ID NO:11 (cDNA for INSR; insulin receptor), SEQ ID NO:13 (cDNA for KIT; c-kit), SEQ ID NO:25 (LOX cDNA for lysyl oxidase preprotein); SEQ ID NO:27 (NOV cDNA for nov precursor), and SEQ ID NO:29 (ANGPTL2 cDNA for angiopoietin-like 2 precursor). Preferably, such antisense molecules are PMO (phosphorodiamidate morpholino Oligomers) antisense molecules.

Thus, the present invention includes nucleic acids that hybridize under stringent hybridization conditions, as defined below, to all or a portion of the validated KHSV-induced cellular gene sequences represented by the cDNA sequences of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29, or the complements thereof. The hybridizing portion of the hybridizing nucleic acids is typically at least 10, 15, 20, 25, 30 or 35 nucleotides in length. Preferably, the hybridizing portion of the hybridizing nucleic acid is at least 80%, at least 95%, or at least 98% identical to the sequence of a portion or all of the cDNA sequences of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29, or to the complements thereof.

Hybridizing nucleic acids of the type described herein can be used, for example, as an inventive therapeutic modulator of KSHV-induced gene expression, a cloning probe, a primer (*e.g.*, a PCR primer), or a diagnostic and/or prognostic probe or primer. Preferably, hybridization of the oligonucleotide probe to a nucleic acid sample is performed under stringent conditions. Nucleic acid duplex or hybrid stability is expressed as the melting temperature or Tm, which is the temperature at which a probe dissociates from a target DNA. This melting temperature is used to define the required stringency conditions.

For sequences that are related and substantially identical to the probe, rather than identical, it is useful to first establish the lowest temperature at which only homologous hybridization occurs with a particular concentration of salt (*e.g.*, SSC or SSPE). Then, assuming that 1% mismatching results in a 1°C decrease in the Tm, the temperature of the final wash in the hybridization reaction is reduced accordingly (for example, if sequences having > 95% identity with the probe are sought, the final wash temperature is decreased by 5°C). In practice, the change in Tm can be between 0.5°C and 1.5°C per 1% mismatch.

Stringent conditions, as defined herein, involve hybridizing at 68°C in 5x SSC/5x Denhardt's solution/1.0% SDS, and washing in 0.2x SSC/0.1% SDS at room temperature, or involve the art-recognized equivalent thereof. Moderately stringent conditions, as defined herein, involve including washing in 3x SSC at 42°C, or the art-recognized equivalent thereof. The parameters of salt concentration and temperature can be varied to achieve the optimal level of identity between the probe and the target nucleic acid. Guidance regarding such conditions is available in the art, for example, by Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y.; and Ausubel et al. (eds.), 1995, Current Protocols in Molecular Biology, (John Wiley & Sons; N.Y.) at Unit 2.10.

Antisense molecules preferably comprise at least 20, or at least 25, and preferably less than about 35 consecutive complementary nucleotides of, or hybridize under stringent conditions to at least one of the nucleic acid sequences from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29. Preferably, such antisense molecules are PMO antisense molecules. Preferred representative antisense molecules are provided herein as:

SEQ ID NO:15	(RDC-1)	5'-GAAGAGATGCAGATCCATCGTTCTG-3');
SEQ ID NO:16	(IGFBP2)	5'-GGCAGCCCACTCTCTCGGCAGCATG-3');
SEQ ID NO:17	(FLJ14103)	5'-GGCTCCATCTTGGGCTCTGGGCTCC-3');
SEQ ID NO:18	(KIAA0367)	5'-GTCAGTTTACTCATGTCATCTATTG-3');
SEQ ID NO:19	(Neuritin)	5'-TTAACTCCCATCCTACGTTTAGTCA-3');
SEQ ID NO:20	(INSR)	5'-GGGTCTCCTCGGATCAGGCGCG-3');
SEQ ID NO:21	(KIT)	5'-CGCCTCTCATCGCGGTAGCTGCG-3');
SEQ ID NO:31	(LOX)	5'-GGAGCACGGTCCAGGCGAAGCGCAT-3');
SEQ ID NO:32	(NOV)	5'-AGCTCGTGCTCTGCACACTCTGCAT-3');

and

SEQ ID NO:33	(ANGPTL2)	5'- AGCATGTCACGCACAGTGGCCTCAT-3').

Preferably, these antisense molecules are PMO antisense molecules.

Even more preferably, representative antisense molecules are provided herein as SEQ ID NOS:15, 16, 17, 19, 21, 31, 32 and 33, and these antisense molecules are preferably PMO antisense molecules.

The invention further provides a ribozyme capable of specifically cleaving at least one RNA specific to RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT, LOX, NOV and ANGPTL2, and a pharmaceutical composition comprising the ribozyme.

The invention also provides small molecule modulators of KSHV-induced gene expression, wherein particular modulators are inhibitors capable of reducing the expression of at

least one KSHV-induced genes, reducing or preventing the expression of mRNA from at least one KSHV-induced gene, or reducing the biological activity of at least one KSHV-induced gene product. Preferably, the KSHV-induced gene is selected from the group consisting of RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT, LOX, NOV and ANGPTL2.

Compositions. Further embodiments provide compositions that comprise one or more modulators of KSHV-induced gene expression (or modulators of biological activity of KSHV-induced gene products) in a pharmaceutically acceptable carrier or diluent.

Particular embodiments provide a pharmaceutical composition for inhibiting KSHV-induced gene expression, comprising an antisense oligonucleotide according to the invention in a mixture with a pharmaceutically acceptable carrier or diluent.

Further provided is a composition comprising a therapeutically effective amount of an inhibitor of a KSHV-induced gene product (*e.g.*, protein) in a pharmaceutically acceptable carrier. In certain embodiments, the composition comprises two or more KSHV-induced gene product inhibitors. Preferably, the KSHV-induced gene product is selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 26, 28 and 30, and combinations thereof, corresponding to RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT, Lysyl Oxidase precursor (LOX), nov precursor (NOV), angiopoietin-like 2 precursor (ANGPTL2), and combinations thereof, respectively.

In particular composition embodiments, the KSHV-induced gene inhibitor is an antisense molecule, and in specific embodiments the antisense molecule or the complement thereof comprises at least 10, 15, 20 or 25 consecutive nucleic acids of, or hybridizes under stringent conditions to at least one of the nucleic acid sequences from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29. Preferably, such antisense molecules are PMO antisense molecules. Preferably, the antisense molecule comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS:15-21 and SEQ ID NOS:31-33. Preferably, the antisense molecules comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS:15, 16, 17, 19, 21, 31, 32 and 33.

Methods and uses. Particular embodiments of the present invention provide methods of modulating KSHV-induced gene expression or biological activity of KSHV-induced gene products in KSHV-infected cells.

The invention provides a method of inhibiting the expression of KSHV-induced cellular genes in human cells or tissues comprising contacting the cells or tissues *in vivo* (also *ex vivo*, or *in vitro*) with an antisense compound or a ribozyme of 10 to 35 nucleotides in length targeted to a nucleic acid molecule encoding a KSHV-induced gene product so that expression of the human

KSHV-induced gene product is inhibited. Preferably, the KSHV-induced gene is selected from the group consisting of RDC-1 (GPCR RDC1), IGFBP2 (insulin-like growth factor binding protein 2), FLJ14103, KIAA0367, Neuritin, INSR (insulin receptor), KIT, Lysyl Oxidase precursor (LOX), nov precursor (NOV), angiopoietin-like 2 precursor (ANGPTL2), and combinations thereof. Preferably, the antisense compounds are PMOs.

The invention additionally provides a method of modulating growth of cancer cells comprising contacting the cancer cells *in vivo* (also *ex vivo*, or *in vitro*) with an inventive antisense compound or ribozyme of 10 to 35 nucleotides in length targeted to a nucleic acid molecule encoding a KSHV-induced gene product so that expression of the human KSHV-induced gene product is inhibited.

The invention provides for the use of a modulator of KSHV-induced gene expression according to the invention to prepare a medicament for modulating cell proliferation and/or phenotype.

Additional embodiments provide a method of inhibiting KSHV-induced gene expression or encoded biological activity in a mammalian cell, comprising administering to the cell an inhibitor of KSHV-induced gene expression (or of encoded biological activity), and in a specific embodiment of the method, the inhibitor is a target gene-specific antisense molecule. Preferably, the antisense molecule is a PMO antisense molecule. Preferably, the antisense molecules comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS:15-21 and SEQ ID NOS:31-33.

The invention also provides a method of inhibiting KSHV-induced gene expression in a subject, comprising administering to said subject, in a pharmaceutically effective vehicle, an amount of an antisense oligonucleotide which is effective to specifically hybridize to all or part of a selected target nucleic acid sequence derived from said KSHV-induced gene. In preferred embodiments of this method, the target-specific antisense oligonucleotide is selected from the group consisting of SEQ ID NOS:15-21 and SEQ ID NOS:31-33. Preferably, the antisense oligonucleotide is selected from the group consisting of SEQ ID NOS:15, 16, 17, 19, 21, 31, 32 and 33. Preferably the antisense oligonucleotides are PMO antisense compounds.

The invention further provides a method of treating KSHV-related neoplastic disease, comprising administering to a mammalian cell a modulator of KSHV-induced gene expression such that the neoplastic disease is reduced in severity.

As discussed herein below, additional embodiments provide screening assays for identification of compounds useful to modulate KSHV infection, comprising: contacting KSHV-infected cells with a test agent; measuring, using a suitable assay, expression of at least one

validated KSHV-induced cellular gene sequence; and determining whether the test agent inhibits said validated gene expression relative to control cells not contacted with the test agent, whereby agents that inhibit said validated gene expression are identified as compounds useful to modulate KSHV infection.

Preferably, expression of at least one validated KSHV-induced cellular gene sequence is expression of respective mRNA, or expression of the protein encoded thereby.

Preferably, the at least one validated KSHV-induced cellular gene sequence is selected from the cDNA and protein sequence group consisting of RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT, Lysyl Oxidase precursor (LOX), nov precursor (NOV), angiopoietin-like 2 precursor (ANGPTL2), and combinations thereof (*i.e.*, consisting of SEQ ID NOS:1-14 and SEQ ID NOS:25-30).

Preferably, agents that inhibit said validated gene expression are further tested for the ability to modulate KSHV-mediated effects on cellular proliferation and/or phenotype.

Further embodiments provide diagnostic or prognostic assays for KSHV infection comprising: obtaining a cell sample from a subject suspected of having KSHV; measuring expression of at least one validated KSHV-inducible cellular gene sequence; and determining whether expression of the at least one validated gene is induced relative to non-KSHV-infected control cells, whereby a diagnosis is afforded.

Preferably, the at least one validated KSHV-inducible cellular gene is selected from the cDNA and protein sequence group consisting of RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT, Lysyl Oxidase precursor (LOX), nov precursor (NOV), angiopoietin-like 2 precursor (ANGPTL2), and combinations thereof (*i.e.*, consisting of SEQ ID NOS:1-14 and SEQ ID NOS:25-30).

Preferably, measuring said expression is of two or more validated KSHV-inducible cellular gene sequences. Preferably, measurement of said expression is by use of high-throughput microarray methods.

Polynucleotides and expression vectors. Particular embodiments provide an isolated polynucleotide with a sequence comprising a transcriptional initiation region and a sequence encoding a KSHV-induced gene-specific antisense oligonucleotide at least 10, 15, 20 or 25 nucleotides in length, and a recombinant vector comprising this polynucleotide (*e.g.*, expression vector). Preferably, the antisense oligonucleotide of said polynucleotide comprises a sequence selected from the group consisting of SEQ ID NOS:15-21 and SEQ ID NOS:31-33. Preferably, the transcriptional initiation region is a strong constitutively expressed mammalian pol III-or pol II-specific promoter, or a viral promoter.

Additional and Preferred Oligonucleotide Modulators

Included within the scope of the invention are oligonucleotides capable of hybridizing with KSHV-induced gene DNA or RNA, referred to herein as the 'target' polynucleotide. An oligonucleotide need not be 100% complementary to the target polynucleotide, as long as specific hybridization is achieved. The degree of hybridization to be achieved is that which interferes with the normal function of the target polynucleotide, be it transcription, translation, pairing with a complementary sequence, or binding with another biological component such as a protein. An antisense oligonucleotide, including a preferred PMO antisense oligonucleotide, can interfere with DNA replication and transcription, and it can interfere with RNA translocation, translation, splicing, and catalytic activity.

The invention includes within its scope any oligonucleotide of about 10 to about 35 nucleotides in length, including variations as described herein, wherein the oligonucleotide hybridizes to a KHSV-induced target sequence, including DNA or mRNA, such that an effect on the normal function of the polynucleotide is achieved. The oligonucleotide can be, for example, 10, 15, 20, 22, 23, 25, 30 or 35 nucleotides in length. Oligonucleotides larger than 35 nucleotides are also contemplated within the scope of the present invention, and may for example, correspond in length to a complete target cDNA (*i.e.*, mRNA) sequence, or to a significant or substantial portion thereof.

Antisense oligonucleotides. As described above, preferred antisense molecules are represented by SEQ ID NOS:15-21 and SEQ ID NOS:31-33.

Examples of representative preferred antisense compounds useful in the invention are based on SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27, 29, and SEQ ID NOS:15-21 and 31-33, and include oligonucleotides containing modified backbones or non-natural internucleoside linkages. Oligonucleotides having modified backbones include those retaining a phosphorus atom in the backbone, and those that do not have a phosphorus atom in the backbone.

Preferred modified oligonucleotide backbones include phosphorothioates or phosphorodithioate, chiral phosphorothioates, phosphotriesters and alkyl phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including methylphosphonates, 3′-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoroamidates or phosphordiamidates, including 3′-amino phosphoroamidate and aminoalkylphosphoroamidates, and phosphorodiamidate morpholino oligomers (PMOs), thiophosphoroamidates, phosphoramidothioates, thioalkylphosphonates,

thionoalkylphosphotriesters, and boranophosphates having normal 3′-5′ linkages, 2′-5′ linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3′-5′ to 5′-3′ or 2′-5′ to 5′-2′. Various salts, mixed salts and free acid forms are also included.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to arabinose, 2-fluoroarabinose, xylulose, hexose and 2′-O-methyl sugar moieties.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine (*see also* U.S. 5,958,773 and patents disclosed therein).

Examples of inventive antisense oligonucleotides of length X (in nucleotides), as indicated by polynucleotide positions with reference to, *e.g.*, SEQ ID NO:1, include those corresponding to sets of consecutively overlapping oligonucleotides of length X, where the oligonucleotides within each consecutively overlapping set (corresponding to a given X value) are defined as the finite set of Z oligonucleotides from nucleotide positions:

n to (n + (X-1));

where n=1, 2, 3,...(Y-(X-1));

where Y equals the length (nucleotides or base pairs) of SEQ ID NO:1 (2,035);

where X equals the common length (in nucleotides) of each oligonucleotide in the set (*e.g.*, X=20 for a set of consecutively overlapping 20-mers); and

where the number (Z) of consecutively overlapping oligomers of length X for a given SEQ ID NO of length Y is equal to Y-(X-1). For example Z=2,035-19=2,016 for SEQ ID NO:1, where X=20.

Examples of inventive 20-mer oligonucleotides include the following set of 2,016 oligomers, indicated by polynucleotide positions with reference to SEQ ID NO:1 (RDC-1 cDNA):

1-20, 2-21, 3-22, 4-23, 5-24, ...2014-2033, 2015-2034 and 2016-2035.

Likewise, examples of 25-mer oligonucleotides include the following set of 2,011 oligomers, indicated by polynucleotide positions with reference to SEQ ID NO:1:

1-25, 2-26, 3-27, 4-28, 5-29, ...2009-2033, 2010-2034 and 2011-2035.

The present invention encompasses, for *each* validated target sequence (*e.g.*, for SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29), multiple consecutively overlapping sets of oligonucleotides or modified oligonucleotides of length X, where, *e.g.*, X= 10, 20, 22, 23, 25, 30 or 35 nucleotides.

Preferred sets of such oligonucleotides or modified oligonucleotides of length X are those consecutively overlapping sets of oligomers corresponding to SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29. Included in these preferred sets are the preferred oligomers corresponding to SEQ ID NOS:15-21 and SEQ ID NOS:31-33.

The antisense oligonucleotides of the invention can also be modified by chemically linking the oligonucleotide to one or more moieties or conjugates to enhance the activity, cellular distribution, or cellular uptake of the antisense oligonucleotide. Such moieties or conjugates include lipids such as cholesterol, cholic acid, thioether, aliphatic chains, phospholipids, polyamines, polyethylene glycol (PEG), palmityl moieties, and others as disclosed in, for example, United States Patent Numbers 5,514,758, 5,565,552, 5,567,810, 5,574,142, 5,585,481, 5,587,371, 5,597,696 and 5,958,773. Thus, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating or modulating transport across the cell membrane (Letsinger et al., *Proc. Natl. Acad. Sci. USA* 86:6553-6556, 1989; Lemaitre et al., *Proc. Natl. Acad. Sci. USA* 84:648-652, 1987; PCT WO88/09810, published Dec. 15, 1988) or the blood-brain barrier (PCT WO89/10134, published Apr. 25, 1988), or the nuclear membrane, and may include hybridization-triggered cleavage agents (Krol et al., *BioTechniques* 6:958-976, 1988) or intercalating agents (Zon, *Pharm. Res.* 5:539-549, 1988). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization-triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

Chimeric antisense oligonucleotides are also within the scope of the invention, and can be prepared from the present inventive oligonucleotides using the methods described in, for example, United States Patent Numbers 5,013,830, 5,149,797, 5,403,711, 5,491,133, 5,565,350, 5,652,355, 5,700,922 and 5,958,773.

Preferred antisense oligonucleotides in addition to those of SEQ ID NOS:15-21 are selected by routine experimentation using, for example, assays described in the present Examples. Although the inventors are not bound by a particular mechanism of action, it is believed that the antisense oligonucleotides achieve an inhibitory effect by binding to a complementary region of the target polynucleotide within the cell using Watson-Crick base pairing. Where the target polynucleotide is RNA, experimental evidence indicates that the RNA component of the hybrid is cleaved by RNase H (Giles, R.V. et al., *Nuc. Acids Res.* (1995) *23*:954-961; U.S. Patent No. 6,001,653). Generally, a hybrid containing 10 base pairs is of sufficient length to serve as a substrate for RNase H. However, to achieve specificity of binding, it is preferable to use an antisense molecule of at least 17 nucleotides, as a sequence of this length is likely to be unique among human genes.

Antisense approaches comprise the design of oligonucleotides (either DNA or RNA) that are complementary to the target gene sequence (*e.g.*, mRNA). The antisense oligonucleotides bind to the complementary mRNA transcripts and prevent translation. Absolute complementarily, although preferred, is not required. A sequence "complementary" to a portion or region of the target mRNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize depends on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA are accommodated without compromising stable duplex (or triplex, as the case may be) formation. One skilled in the art ascertains a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

As disclosed in U.S. Patent No. 5,998,383, incorporated herein by reference, the oligonucleotide is selected such that the sequence exhibits suitable energy related characteristics important for oligonucleotide duplex formation with their complementary targets, and shows a low potential for self-dimerization or self-complementation (Anazodo et al., *Biochem. Biophys. Res. Commun.* (1996) *229*:305-309). The computer program OLIGO (Primer Analysis Software, Version 3.4), is used to determined antisense sequence melting temperature, free

energy properties, and to estimate potential self-dimer formation and self-complementarity properties. The program allows the determination of a qualitative estimation of these two parameters (potential self-dimer formation and self-complementary) and provides an indication of "no potential" or "some potential" or "essentially complete potential." Preferably, segments of validated KSHV-induced gene sequences are selected that have estimates of no potential in these parameters. However, segments that have "some potential" in one of the categories nonetheless can have utility, and a balance of the parameters is routinely used in the selection.

While antisense nucleotides complementary to the coding region sequence of a mRNA are used in accordance with the invention, those complementary to the transcribed, untranslated region, or translational initiation site region are sometimes preferred. Oligonucleotides that are complementary to the 5' end of the message, *e.g.*, the 5'-untranslated sequence (up to and including the AUG initiation codon), frequently work most efficiently at inhibiting translation. However, sequences complementary to the 3'-untranslated sequences, or other regions of mRNAs are also effective at inhibiting translation of mRNAs (*see e.g.*, Wagner, *Nature* 372:333-335, 1994). In the antisense art a certain degree of routine experimentation is required to select optimal antisense molecules for particular targets. To be effective, the antisense molecule preferably is targeted to an accessible, or exposed, portion of the target RNA molecule. Although in some cases information is available about the structure of target mRNA molecules, the current approach to inhibition using antisense is via experimentation.

Such experimentation can be performed routinely by transfecting or loading cells with an antisense oligonucleotide, followed by measurement of messenger RNA (mRNA) levels in the treated and control cells by reverse transcription of the mRNA and assaying of respective cDNA levels. Measuring the specificity of antisense activity by assaying and analyzing cDNA levels is an art-recognized method of validating antisense results. Routinely, RNA from treated and control cells is reverse-transcribed and the resulting cDNA populations are analyzed (Branch, A. D., *T.I.B.S*.(1998) *23*:45-50).

According to the present invention, antisense efficacy can be alternately determined by measuring the biological effects on cell growth, phenotype or viability as is known in the art, and as shown in the present Examples. According to the present invention, cultures of KSHV-infected DMVEC were loaded with inventive oligonucleotides designed to target KSHV-induced gene sequences. Preferred representative antisense oligonucleotides correspond to SEQ ID NOS:15-21. The effects of such loading on cellular proliferation and/or phenotype were measured. Specifically, SEQ ID NOS:15-21 caused dramatic decreases in cell proliferation and inhibited/reverted spindle cell formation, both hallmarks of *in vivo* KSHV-related cancer.

Ribozymes. Modulators of KSHV-induced gene expression may be ribozymes. A ribozyme is an RNA molecule that specifically cleaves RNA substrates, such as mRNA, resulting in specific inhibition or interference with cellular gene expression. As used herein, the term ribozymes includes RNA molecules that contain antisense sequences for specific recognition, and an RNA-cleaving enzymatic activity. The catalytic strand cleaves a specific site in a target RNA at greater than stoichiometric concentration. Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the target mRNA (*i.e.*, to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts).

A wide variety of ribozymes may be utilized within the context of the present invention, including for example, the hammerhead ribozyme (for example, as described by Forster and Symons, *Cell* (1987) *48*:211-220; Haseloff and Gerlach, *Nature* (1988) *328*:596-600; Walbot and Bruening, *Nature* (1988) *334*:196; Haseloff and Gerlach, *Nature* (1988) *334*:585); the hairpin ribozyme (for example, as described by Haseloff et al., U.S. Patent No. 5,254,678, issued October 19, 1993 and Hempel et al., European Patent Publication No. 0 360 257, published March 26, 1990); and Tetrahymena ribosomal RNA-based ribozymes (*see* Cech et al., U.S. Patent No. 4,987,071). The Cech-type ribozymes have an eight-base pair active site that hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. Ribozymes of the present invention typically consist of RNA, but may also be composed of DNA, nucleic acid analogs (*e.g.*, phosphorothioates), or chimerics thereof (*e.g.*, DNA/RNA/RNA).

Ribozymes can be targeted to any RNA transcript and can catalytically cleave such transcripts (*see, e.g.*, U.S. Patent No. 5,272,262; U.S. Patent No. 5,144,019; and U.S. Patent Nos. 5,168,053, 5,180,818, 5,116,742 and 5,093,246 to Cech et al.). According to certain embodiments of the invention, any such KSHV-induced gene sequence-specific ribozyme, or a nucleic acid encoding such a ribozyme, may be delivered to a host cell to effect inhibition of KSHV-induced gene expression. Ribozymes and the like may therefore be delivered to the host cells by DNA encoding the ribozyme linked to a eukaryotic promoter (*e.g.*, a strong constitutively expressed pol III- or pol II-specific promoter), or a eukaryotic viral promoter, such that upon introduction into the nucleus, the ribozyme will be directly transcribed.

Triple-helix formation. Alternatively, validated KSHV-induced gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of

the target gene (*e.g.*, respective promoter and/or enhancers) to form triple helical structures that prevent transcription of the target gene (*see, e.g.*, Helen, *Anticancer Drug Des.*, 6:569-84, 1991; Helene et al., *Ann, N.Y. Acad. Sci.*, 660:27-36, 1992; and Maher, *Bioassays* 14:807-15, 1992).

siRNA. The invention, in particular aspects, contemplates introduction of RNA with partial or fully double-stranded character into the cell or into the extracellular environment. According to the present invention, inhibition is specific to the particular validated KSHV-induced cellular gene expression product in that a nucleotide sequence from a portion of the validated sequence is chosen to produce inhibitory RNA. This process is effective in producing inhibition (partial or complete), and is validated gene-specific. In particular embodiments, the target cell containing the validate gene may be a human cell subject to infection by KSHV (or cell-lines derived therefrom). Methods of preparing and using siRNA are generally disclosed in U.S. Patent 6,506,559, incorporated herein by reference (*see also* reviews by Milhavet et al., *Pharmacological Reviews* 55:629-648, 2003; and Gitlin et al., *J. Virol.* 77:7159-7165, 2003; incorporated herein by reference).

The siRNA may comprise one or more strands of polymerized ribonucleotide, and may include modifications to either the phosphate-sugar backbone or the nucleoside. For example, the phosphodiester linkages of natural RNA may be modified to include at least one of a nitrogen or sulfur heteroatom. Modifications in RNA structure may be tailored to allow specific genetic inhibition while avoiding a general panic response in some organisms which is generated by dsRNA. Likewise, bases may be modified to block the activity of adenosine deaminase. RNA may be produced enzymatically or by partial/total organic synthesis, any modified ribonucleotide can be introduced by *in vitro* enzymatic or organic synthesis.

The double-stranded structure may be formed by a single self-complementary RNA strand or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. The RNA may be introduced in an amount which allows delivery of at least one copy per cell. Higher doses of double-stranded material may yield more effective inhibition. Inhibition is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition. Nucleic acid containing a nucleotide sequence identical to a portion of the validated gene sequence is preferred for inhibition. RNA sequences with insertions, deletions, and single point mutations relative to the target sequence have also been found to be effective for inhibition. Sequence identity may be optimized by alignment algorithms known in the art and calculating the percent difference between the nucleotide sequences. Alternatively, the duplex region of the RNA may be defined

functionally as a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript.

RNA may be synthesized either *in vivo* or *in vitro*. Endogenous RNA polymerase of the cell may mediate transcription *in vivo*, or cloned RNA polymerase can be used for transcription *in vivo* or *in vitro*. For transcription from a transgene *in vivo* or an expression construct, a regulatory region may be used to transcribe the RNA strand (or strands).

For siRNA (RNAi), the RNA may be directly introduced into the cell (*i.e.*, intracellularly); or introduced extracellularly into a cavity, interstitial space, into the circulation of an organism, introduced orally, or may be introduced by bathing an organism in a solution containing RNA. Methods for oral introduction include direct mixing of RNA with food of the organism, as well as engineered approaches in which a species that is used as food is engineered to express a RNA, then fed to the organism to be affected. Physical methods of introducing nucleic acids include injection directly into the cell or extracellular injection into the organism of an RNA solution.

Inhibition of gene expression refers to the absence (or observable decrease) in the level of protein and/or mRNA product from a validated gene target. Specificity refers to the ability to inhibit the target gene without manifest effects on other genes of the cell. The consequences of inhibition can be confirmed by examination of the outward properties of the cell or organism or by biochemical techniques such as RNA solution hybridization, nuclease protection, Northern hybridization, reverse transcription, gene expression monitoring with a microarray, antibody binding, enzyme linked immunosorbent assay (ELISA), Western blotting, radioimmunoassay (RIA), other immunoassays, fluorescence activated cell analysis (FACS), and KSHV viral infection and/or replication, inhibition of KSHV-induced proliferation, or inhibition of KSHV induced cellular phenotype, as described herein. For RNA-mediated inhibition in a cell line or whole organism, gene expression is conveniently assayed by use of a reporter or drug resistance gene whose protein product is easily assayed. Many such reporter genes are known in the art.

The phosphodiester linkages of natural RNA may be modified to include at least one of a nitrogen or sulfur heteroatom. Modifications in RNA structure may be tailored to allow specific genetic inhibition while avoiding a general panic response in some organisms which is generated by dsRNA. Likewise, bases may be modified to block the activity of adenosine deaminase. RNA may be produced enzymatically or by partial/total organic synthesis, any modified ribonucleotide can be introduced by *in vitro* enzymatic or organic synthesis.

RNA containing a nucleotide sequences identical to a portion of a particular validated gene sequence are preferred for inhibition. RNA sequences with insertions, deletions, and single

point mutations relative to the target sequence may be effective for inhibition. Sequence identity may optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, Sequence Analysis Primer, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (*e.g.*, University of Wisconsin Genetic Computing Group). Greater than 90% sequence identity, or even 100% sequence identity, between the inhibitory RNA and the portion of particular validated gene sequence is preferred. Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the particular validated gene transcript (*e.g.*, 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C. or 70°C. hybridization for 12-16 hours; followed by washing). The length of the identical nucleotide sequences may be at least 20, 25, 50, 100, 200, 300 or 400 bases. Preferably, wherein the siRNA agent specific for a *validated* KSHV-induced cellular gene sequence comprises a nucleic acid sequence of, *e.g.*, at least 9, at least 15, at least 18, or at least 20 contiguous bases in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27, 29, and sequences complementary thereto.

A 100% sequence identity between the RNA and a particular validated gene sequence is not required to practice the present invention. Thus the methods have the advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence.

Particular validated gene sequence siRNA may be synthesized by art-recognized methods either *in vivo* or *in vitro*. Endogenous RNA polymerase of the cell may mediate transcription *in vivo*, or cloned RNA polymerase can be used for transcription in vivo or *in vitro*. For transcription from a transgene *in vivo* or an expression construct, a regulatory region (e.g., promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the RNA strand (or strands). Inhibition may be targeted by specific transcription in an organ, tissue, or cell type; stimulation of an environmental condition (*e.g.*, infection, stress, temperature, chemical inducers); and/or engineering transcription at a developmental stage or age. The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus.

RNA may be chemically or enzymatically synthesized by manual or automated reactions. The RNA may be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (*e.g.*, T3, T7, SP6). The use and production of an expression construct are known in

the art (*e.g.*, WO 97/32016; U.S. Pat. Nos: 5,593,874, 5,698,425, 5,712,135, 5,789,214, and 5,804,693; and the references cited therein). If synthesized chemically or by *in vitro* enzymatic synthesis, the RNA may be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, the RNA may be used with no or a minimum of purification to avoid losses due to sample processing. The RNA may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

siRNA may be directly introduced into the cell (*i.e.*, intracellularly); or introduced extracellularly into a cavity, interstitial space, into the circulation of an organism, introduced orally, or may be introduced by bathing an organism in a solution containing the RNA. Methods for oral introduction include direct mixing of the RNA with food of the organism, as well as engineered approaches in which a species that is used as food is engineered to express the RNA, then fed to the organism to be affected. For example, the RNA may be sprayed onto a plant or a plant may be genetically engineered to express the RNA in an amount sufficient to kill some or all of a pathogen known to infect the plant. Physical methods of introducing nucleic acids, for example, injection directly into the cell or extracellular injection into the organism, may also be used. Vascular or extravascular circulation, the blood or lymph system, and the cerebrospinal fluid are sites where the RNA may be introduced. A transgenic organism that expresses RNA from a recombinant construct may be produced by introducing the construct into a zygote, an embryonic stem cell, or another multipotent cell derived from the appropriate organism.

Physical methods of introducing nucleic acids include injection of a solution containing the RNA, bombardment by particles covered by the RNA, soaking the cell or organism in a solution of the RNA, or electroporation of cell membranes in the presence of the RNA. A viral construct packaged into a viral particle would accomplish both efficient introduction of an expression construct into the cell and transcription of RNA encoded by the expression construct. Other methods known in the art for introducing nucleic acids to cells may be used, such as lipid-mediated carrier transport, chemical-mediated transport, such as calcium phosphate, and the like. Thus the RNA may be introduced along with components that perform one or more of the following activities: enhance RNA uptake by the cell, promote annealing of the duplex strands, stabilize the annealed strands, or other-wise increase inhibition of the target gene.

The siRNA may be used alone or as a component of a kit having at least one of the reagents necessary to carry out the *in vitro* or *in vivo* introduction of RNA to test samples or subjects. Preferred components are the dsRNA and a vehicle that promotes introduction of the

dsRNA. Such a kit may also include instructions to allow a user of the kit to practice the invention.

Suitable injection mixes are constructed so animals receive an average of 0.5×10^6 to 1.0×10^6 molecules of RNA. For comparisons of sense, antisense, and dsRNA activities, injections are compared with equal masses of RNA (*i.e.*, dsRNA at half the molar concentration of the single strands). Numbers of molecules injected per adult are given as rough approximations based on concentration of RNA in the injected material (estimated from ethidium bromide staining) and injection volume (estimated from visible displacement at the site of injection). A variability of several-fold in injection volume between individual animals is possible.

Proteins and Polypeptides

In addition to the antisense molecules and ribozymes disclosed herein, inventive modulators of KSHV-induced gene expression also include proteins or polypeptides that are effective in either reducing validated KSHV-induced cellular gene expression or in decreasing one or more of the respective biological activities encoded thereby. A variety of art-recognized methods are used by the skilled artisan, through routine experimentation, to rapidly identify such modulators of KSHV-induced gene expression. The present invention is not limited by the following exemplary methodologies.

Inhibitors of KSHV-induced biological activities encompass those proteins and/or polypeptides that interfere with said biological activities. Such interference may occur through direct interaction with active domains of the proteins of validated gene targets, or indirectly through non- or un-competitive inhibition such as via binding to an allosteric site. Accordingly, available methods for identifying proteins and/or polypeptides that bind to proteins of validated gene targets may be employed to identify lead compounds that may, through the methodology disclosed herein, be characterized for their inhibitory activity.

Methods for detecting and analyzing protein-protein interactions are described in the art, and are thus available to skilled artisans (*reviewed in* Phizicky, E.M. et al., *Microbiological Reviews* (1995) *59*:94-123 incorporated herein by reference. Such methods include, but are not limited to physical methods such as, *e.g.*, protein affinity chromatography, affinity blotting, immunoprecipitation and cross-linking as well as library-based methods such as, *e.g.*, protein probing, phage display and two-hybrid screening. Other methods that may be employed to identify protein-protein interactions include genetic methods such as use of extragenic suppressors, synthetic lethal effects and unlinked noncomplementation. Exemplary methods are described in further detail below.

Inventive inhibitors of proteins of validated gene targets (validated proteins) may be identified through biological screening assays that rely on the direct interaction between the a validated protein (*e.g.*, SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 26, 28 and 30) and a panel or library of potential inhibitor proteins. Biological screening methodologies, including the various "n-hybrid technologies," are described in, for example, Vidal, M. et al., *Nucl. Acids Res.* (1999) *27*(4):919-929; Frederickson, R.M., *Curr. Opin. Biotechnol.* (1998) *9*(1):90-6; Brachmann, R.K. et al., *Curr. Opin. Biotechnol.* (1997) *8*(5):561-568; and White, M.A., *Proc. Natl. Acad. Sci. U.S.A.* (1996) *93*:10001-10003 each of which is incorporated herein by reference.

The two-hybrid screening methodology may be employed to search new or existing target cDNA libraries for inhibitory proteins. The two-hybrid system is a genetic method that detects protein-protein interactions by virtue of increases in transcription of reporter genes. The system relies on the fact that site-specific transcriptional activators have a DNA-binding domain and a transcriptional activation domain. The DNA-binding domain targets the activation domain to the specific genes to be expressed. Because of the modular nature of transcriptional activators, the DNA-binding domain may be severed from the otherwise covalently linked transcriptional activation domain without loss of activity of either domain. Furthermore, these two domains may be brought into juxtaposition by protein-protein contacts between two proteins unrelated to the transcriptional machinery. Thus, two hybrids are constructed to create a functional system. The first hybrid, *i.e.*, the bait, consists of a transcriptional activator DNA-binding domain fused to a protein of interest (*e.g.*, SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 26, 28 and 30, or fragments thereof). The second hybrid, the target, is created by the fusion of a transcriptional activation domain with a library of proteins or polypeptides. Interaction between the bait protein and a member of the target library results in the juxtaposition of the DNA-binding domain and the transcriptional activation domain and the consequent up-regulation of reporter gene expression.

A variety of two-hybrid based systems are available to the skilled artisan that most commonly employ either the yeast Gal4 or *E. coli* LexA DNA-binding domain (BD) and the yeast Gal4 or herpes simplex virus VP16 transcriptional activation domain. Chien, C.-T. et al., *Proc. Natl. Acad. Sci. U.S.A.* (1991) *88*:9578-9582; Dalton, S. et al., *Cell* (1992) *68*:597-612; Durfee, T.K. et al., *Genes Dev.* (1993) *7*:555-569; Vojtek, A.B. et al., *Cell* (1993) *74*:205-214; and Zervos, A.S. et al., *Cell* (1993) *72*:223-232. Commonly used reporter genes include the *E. coli lacZ* gene as well as selectable yeast genes such as *HIS3* and *LEU2*. Fields, S. et al., *Nature (London)* (1989) *340*:245-246; Durfee, T.K., *supra*; and Zervos, A.S., *supra*. A wide variety of

activation domain libraries is readily available in the art such that the screening for interacting proteins may be performed through routine experimentation.

Suitable bait proteins for the identification of inhibitors of validated proteins are designed based on the validated sequences presented herein as SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 26, 28 and 30. Such bait proteins include either the full-length validated protein, or fragments thereof.

Plasmid vectors, such as, *e.g.*, pBTM116 and pAS2-1, for preparing validated protein bait constructs and target libraries are readily available to the artisan and may be obtained from such commercial sources as, *e.g.*, Clontech (Palo Alto, CA), Invitrogen (Carlsbad, CA) and Stratagene (La Jolla, CA). These plasmid vectors permit the in-frame fusion of cDNAs with the DNA-binding domains as LexA or Gal4BD, respectively.

Validated protein inhibitors of the present invention may alternatively be identified through one of the physical or biochemical methods available in the art for detecting protein-protein interactions.

For example, affinity chromatography may be used to identify potential inhibitors of validated proteins, by virtue of specific retention of such potential inhibitors to validated proteins, or to fragments thereof covalently or non-covalently coupled to a solid matrix such as, *e.g.*, Sepharose beads. The preparation of protein affinity columns is described in, for example, Beeckmans, S. et al., *Eur. J. Biochem.* (1981) *117*:527-535 and Formosa, T. et al., *Methods Enzymol.* (1991) *208*:24-45. Cell lysates containing the full complement of cellular proteins may be passed through a validated protein affinity column. Proteins having a high affinity for the validated protein will be specifically retained under low-salt conditions while the majority of cellular proteins will pass through the column. Such high affinity proteins may be eluted from the immobilized validated protein, or fragment thereof under conditions of high-salt, with chaotropic solvents or with sodium dodecyl sulfate (SDS). In some embodiments, it may be preferred to radiolabel the cells prior to preparing the lysate as an aid in identifying the validated protein-specific binding proteins. Methods for radiolabeling mammalian cells are well known in the art and are provided, *e.g.*, in Sopta, M. et al., *J. Biol. Chem.* (1985) *260*:10353-10360.

Suitable validated proteins for affinity chromatography may be fused to a protein or polypeptide to permit rapid purification on an appropriate affinity resin. For example, a validated protein cDNA may be fused to the coding region for glutathione S-transferase (GST) which facilitates the adsorption of fusion proteins to glutathione-agarose columns. Smith et al., *Gene* (1988) *67*:31-40. Alternatively, fusion proteins may include protein A, which can be purified on columns bearing immunoglobulin G; oligohistidine-containing peptides, which can

be purified on columns bearing Ni^{2+}; the maltose-binding protein, which can be purified on resins containing amylose; and dihydrofolate reductase, which can be purified on methotrexate columns. One such tag suitable for the preparation of validate protein fusion proteins is the epitope for the influenza virus hemagglutinin (HA) against which monoclonal antibodies are readily available and from which antibodies an affinity column may be prepared.

Proteins that are specifically retained on a validated protein affinity column may be identified after subjecting to SDS polyacrylamide gel electrophoresis (SDS-PAGE). Thus, where cells are radiolabeled prior to the preparation of cell lysates and passage through the validated protein affinity column, proteins having high affinity for the particular validate protein may be detected by autoradiography. The identity of particular validated protein-specific binding proteins may be determined by protein sequencing techniques that are readily available to the skilled artisan, such as those described by Mathews, C.K. et al., *Biochemistry*, The Benjamin/Cummings Publishing Company, Inc. pp. 166-170 (1990).

Antibodies or Antibody Fragments

Inhibitors of KSHV-induced gene expression of the present invention include antibodies and/or antibody fragments that are effective in reducing KSHV-induced gene expression and/or reducing the biological activity encoded thereby. Suitable antibodies may be monoclonal, polyclonal or humanized monoclonal antibodies. Antibodies may be derived by conventional hybridoma based methodology, from antisera isolated from validated protein inoculated animals or through recombinant DNA technology. Alternatively, inventive antibodies or antibody fragments may be identified *in vitro* by use of one or more of the readily available phage display libraries. Exemplary methods are disclosed herein.

In one embodiment of the present invention, validated protein inhibitors are monoclonal antibodies that may be produced as follows. Validated proteins (SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 26, 28 and 30) may be produced, for example, by expression of the respective cDNAs (SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29, respectively) in a baculovirus based system. By this method, validated protein cDNAs (SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29) or epitope-bearing fragments thereof are ligated into a suitable plasmid vector that is subsequently used to transfect Sf9 cells to facilitate protein production. In addition, it may be advantageous to incorporate an epitope tag or other moiety to facilitate affinity purification of the validated protein. Clones of Sf9 cells expressing a particular validated protein are identified, *e.g.*, by enzyme-linked immunosorbant assay (ELISA), lysates are prepared and the validated protein purified by affinity chromatography. The purified validated protein is, for example, injected

intraperitoneally, into BALB/c mice to induce antibody production. It may be advantageous to add an adjuvant, such as Freund's adjuvant, to increase the resulting immune response.

Serum is tested for the production of specific antibodies, and spleen cells from animals having a positive specific antibody titer are used for cell fusions with myeloma cells to generate hybridoma clones. Supernatants derived from hybridoma clones are tested for the presence of monoclonal antibodies having specificity against a particular validated protein (*e.g.*, SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 26, 28 and 30, or fragments thereof). For a general description of monoclonal antibody methodology, *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory (1988).

In addition to the baculovirus expression system, other suitable bacterial or yeast expression systems may be employed for the expression of a particular validated protein or polypeptides thereof. As with the baculovirus system, it may be advantageous to utilize one of the commercially available affinity tags to facilitate purification prior to inoculation of the animals. Thus, the a validated protein cDNA or fragment thereof may be isolated by, *e.g.*, agarose gel purification and ligated in frame with a suitable tag protein such as 6-His, glutathione-S-transferase (GST) or other such readily available affinity tag. *See, e.g., Molecular Biotechnology: Principles and Applications of Recombinant DNA*, ASM Press pp. 160-161 (*ed.* Glick, B.R. and Pasternak, J.J. 1998).

In other embodiments of the present invention, inhibitors of validated proteins are humanized anti-validated protein monoclonal antibodies. The phrase "humanized antibody" refers to an antibody derived from a non-human antibody—typically a mouse monoclonal antibody. Alternatively, a humanized antibody may be derived from a chimeric antibody that retains or substantially retains the antigen-binding properties of the parental, non-human, antibody but which exhibits diminished immunogenicity as compared to the parental antibody when administered to humans. The phrase "chimeric antibody," as used herein, refers to an antibody containing sequence derived from two different antibodies (*see, e.g.*, U.S. Patent No. 4,816,567) which typically originate from different species. Most typically, chimeric antibodies comprise human and murine antibody fragments, generally human constant and mouse variable regions.

Because humanized antibodies are far less immunogenic in humans than the parental mouse monoclonal antibodies, they can be used for the treatment of humans with far less risk of anaphylaxis. Thus, these antibodies may be preferred in therapeutic applications that involve *in vivo* administration to a human such as, *e.g.*, use as radiation sensitizers for the treatment of neoplastic disease or use in methods to reduce the side effects of, *e.g.*, cancer therapy.

Humanized antibodies may be achieved by a variety of methods including, for example: (1) grafting the non-human complementarity determining regions (CDRs) onto a human framework and constant region (a process referred to in the art as "humanizing"), or, alternatively, (2) transplanting the entire non-human variable domains, but "cloaking" them with a human-like surface by replacement of surface residues (a process referred to in the art as "veneering"). In the present invention, humanized antibodies will include both "humanized" and "veneered" antibodies. These methods are disclosed in, *e.g.*, Jones et al., *Nature* (1986) *321*:522-525; Morrison et al., *Proc. Natl. Acad. Sci., U.S.A.*, (1984) *81*:6851-6855; Morrison and Oi, *Adv. Immunol.* (1988) *44*:65-92; Verhoeyer et al., *Science* (1988) *239*:1534-1536; Padlan, *Molec. Immun.* (1991) *28*:489-498; Padlan, *Molec. Immunol.* (1994) *31*(3):169-217; and Kettleborough, C.A. et al., *Protein Eng.* (1991) *4*:773-83 each of which is incorporated herein by reference.

The phrase "complementarity determining region" refers to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. *See, e.g.*, Chothia et al., *J. Mol. Biol.* (1987) *196*:901-917; Kabat et al., U.S. Dept. of Health and Human Services NIH Publication No. 91-3242 (1991). The phrase "constant region" refers to the portion of the antibody molecule that confers effector functions. In the present invention, mouse constant regions are substituted by human constant regions. The constant regions of the subject humanized antibodies are derived from human immunoglobulins. The heavy chain constant region can be selected from any of the five isotypes: alpha, delta, epsilon, gamma or mu.

One method of humanizing antibodies comprises aligning the non-human heavy and light chain sequences to human heavy and light chain sequences, selecting and replacing the non-human framework with a human framework based on such alignment, molecular modeling to predict the conformation of the humanized sequence and comparing to the conformation of the parent antibody. This process is followed by repeated back mutation of residues in the CDR region which disturb the structure of the CDRs until the predicted conformation of the humanized sequence model closely approximates the conformation of the non-human CDRs of the parent non-human antibody. Such humanized antibodies may be further derivatized to facilitate uptake and clearance, *e.g.*, via Ashwell receptors (*see, e.g.*, U.S. Patent Nos. 5,530,101 and 5,585,089, both incorporated herein by reference.

Humanized antibodies to a particular validated protein can also be produced using transgenic animals that are engineered to contain human immunoglobulin loci. For example, WO 98/24893 discloses transgenic animals having a human Ig locus wherein the animals do not

produce functional endogenous immunoglobulins due to the inactivation of endogenous heavy and light chain loci. WO 91/10741 also discloses transgenic non-primate mammalian hosts capable of mounting an immune response to an immunogen, wherein the antibodies have primate constant and/or variable regions, and wherein the endogenous immunoglobulin-encoding loci are substituted or inactivated. WO 96/30498 discloses the use of the Cre/Lox system to modify the immunoglobulin locus in a mammal, such as to replace all or a portion of the constant or variable region to form a modified antibody molecule. WO 94/02602 discloses non-human mammalian hosts having inactivated endogenous Ig loci and functional human Ig loci. U.S. Patent No. 5,939,598 discloses methods of making transgenic mice in which the mice lack endogenous heavy claims, and express an exogenous immunoglobulin locus comprising one or more xenogeneic constant regions.

Using a transgenic animal described above, an immune response can be produced to a selected antigenic molecule (*e.g.*, validated protein or fragment thereof), and antibody-producing cells can be removed from the animal and used to produce hybridomas that secrete human monoclonal antibodies. Immunization protocols, adjuvants, and the like are known in the art, and are used in immunization of, for example, a transgenic mouse as described in WO 96/33735. This publication discloses monoclonal antibodies against a variety of antigenic molecules including IL-6, IL-8, TNFα, human CD4, L-selectin, gp39, and tetanus toxin. The monoclonal antibodies can be tested for the ability to inhibit or neutralize the biological activity or physiological effect of the corresponding protein. WO 96/33735 discloses that monoclonal antibodies against IL-8, derived from immune cells of transgenic mice immunized with IL-8, blocked IL-8-induced functions of neutrophils. Human monoclonal antibodies with specificity for the antigen used to immunize transgenic animals are also disclosed in WO 96/34096.

For purposes of the present invention, validated polypeptides and variants thereof are used to immunize a transgenic animal as described above. Monoclonal antibodies are made using methods known in the art, and the specificity of the antibodies is tested using isolated validated polypeptides. The suitability of the antibodies for clinical use is tested by, for example, exposing KSHV-infected DMVEC cells to the antibodies and measuring cell growth and/or phenotypic changes. According to the invention, inhibition of KSHV-induced gene sequence expression using antisense oligonucleotides specific for validated KSHV-induced polynucleotides causes an inhibition of anchorage-independent growth of KSHV-infected DMVEC cells. The antisense oligonucleotides also inhibited spindle cell formation of KSHV-infected DMVEC cells (or caused reversion of the spindle cell phenotype). Human monoclonal antibodies specific for a particular validated protein, or for a variant or fragment thereof can be

tested for their ability to inhibit proliferation, colony growth, or any other biological parameter (*e.g.*, spindle cell formation) indicative of control of tumor growth, migration, or metastasis, particularly tumor cells of epithelial or endothelial origin. Such antibodies would be suitable for pre-clinical and clinical trials as pharmaceutical agents for preventing or controlling growth of cancer cells, including KSHV-related cancer cells.

It will be appreciated that alternative validated protein inhibitor antibodies may be readily obtained by other methods commonly known in the art. One exemplary methodology for identifying antibodies having a high specificity for a particular validated protein is the phage display technology.

Phage display libraries for the production of high-affinity antibodies are described in, for example, Hoogenboom, H.R. et al., *Immunotechnology* (1998) *4*(1):1-20; Hoogenboom, H.R., *Trends Biotechnol.* (1997) *15*:62-70 and McGuinness, B. et al., *Nature Bio. Technol.* (1996) *14*:1149-1154 each of which is incorporated herein by reference. Among the advantages of the phage display technology is the ability to isolate antibodies of human origin that cannot otherwise be easily isolated by conventional hybridoma technology. Furthermore, phage display antibodies may be isolated *in vitro* without relying on an animal's immune system.

Antibody phage display libraries may be accomplished, for example, by the method of McCafferty et al., *Nature* (1990) *348*:552-554 which is incorporated herein by reference. In short, the coding sequence of the antibody variable region is fused to the amino terminus of a phage minor coat protein (pIII). Expression of the antibody variable region-pIII fusion construct results in the antibody's "display" on the phage surface with the corresponding genetic material encompassed within the phage particle.

A validated protein, or fragment thereof suitable for screening a phage library may be obtained by, for example, expression in baculovirus Sf9 cells as described, *supra*. Alternatively, the validated protein coding region may be PCR amplified using primers specific to the desired region of the validated protein. As discussed above, the validated protein may be expressed in *E. coli* or yeast as a fusion with one of the commercially available affinity tags.

The resulting fusion protein may then be adsorbed to a solid matrix, *e.g.*, a tissue culture plate or bead. Phage expressing antibodies having the desired anti-validated protein binding properties may subsequently be isolated by successive panning, in the case of a solid matrix, or by affinity adsorption to a validated protein antigen column. Phage having the desired validated protein inhibitory activities may be reintroduced into bacteria by infection and propagated by standard methods known to those skilled in the art. *See* Hoogenboom, *H.R., Trends Biotechnol., supra* for a review of methods for screening for positive antibody-pIII phage.

Small Molecules and High-throughput Screening (HTS) Assays

As discussed herein, particular embodiments of the present invention provide screening assays for identification of compounds useful to modulate KSHV infection, comprising: contacting KSHV-infected cells with a test agent; measuring, using a suitable assay, expression of at least one validated KSHV-induced cellular gene sequence; and determining whether the test agent inhibits said validated gene expression relative to control cells not contacted with the test agent, whereby agents that inhibit said validated gene expression are identified as compounds useful to modulate KSHV infection.

Preferably, the at least one validated KSHV-induced cellular gene sequence is selected from the cDNA and protein sequence group consisting of RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT, LOX, NOV and ANGPTL2, and combinations thereof (*i.e.*, consisting of SEQ ID NOS:1-14 and SEQ ID NOS:25-30). Preferably, expression of at least one validated KSHV-induced cellular gene sequence is expression of at least one of mRNA, or expression of the protein encoded thereby. Preferably, agents that inhibit said validated gene expression are further tested for the ability to modulate KSHV-mediated effects on cellular proliferation and/or phenotype.

The present invention also provides small molecule modulators that may be readily identified through routine application of high-throughput screening (HTS) methodologies. *Reviewed by* Persidis, A., *Nature Biotechnology* (1998) *16*:488-489. HTS methods generally permit the rapid screening of test compounds, such as small molecules, for therapeutic potential. HTS methodology employs robotic handling of test materials, detection of positive signals and interpretation of data. Such methodologies include, *e.g.*, robotic screening technology using soluble molecules as well as cell-based systems such as the two-hybrid system described in detail above.

A variety of cell line-based HTS methods are available that benefit from their ease of manipulation and clinical relevance of interactions that occur within a cellular context as opposed to in solution. Test compounds are identified via incorporation of radioactivity or through optical assays that rely on absorbance, fluorescence or luminescence as read-outs. *See, e.g.*, Gonzalez, J.E. et al., *Curr. Opin. Biotechnol.* (1998) *9*(6):624-631 incorporated herein by reference.

HTS methodology is employed, *e.g.*, to screen for test compounds that modulate or block one of the biological activities of a validated protein (*i.e.*, a protein encoded by validated KSHV-induced cellular gene expression). For example, a validated protein may be immunoprecipitated

from cells expressing the protein and applied to wells on an assay plate suitable for robotic screening. Individual test compounds are contacted with the immunoprecipitated protein and the effect of each test compound on an activity of the validated protein is assessed. For example, if the particular validated protein has kinase activity, the effect of a particular test compound on the kinase is assessed by, *e.g.*, incubating the corresponding immunopreciped protein in contact with the particular test compound in the presence of γ-^{32}P-ATP in a suitable buffer system, and measuring the incorporation of ^{32}P.

Both small molecule agonists and antagonists of particular validated proteins (SEQ ID NOS:2, 4, 6, 8 10, 12, 14, 26, 28 and 30) are encompassed within the scope of the present invention.

Preferably, KSHV-infected DMVEC are used in inventive screening assays for therapeutic compounds.

Gleevec™, for example, as described by Moses et al., J. Virol. 76:8383-8399, 2002 (*see also* WO0210339A2), is a representative example of a small molecule modulator of c-Kit tyrosine kinase activity that modulates KSHV-induced cellular gene expression. STI 571 (Gleevec™) was designed as an ATP-competitive inhibitor of the Abl tyrosine kinase, and was later shown to be active against c-Kit (Heinrich et al., *Blood* 96:925-932m 2000).

The proliferative response of KSHV-infected DMVEC to exogenous SCF is inhibited by STI 571, where cell viability controls show that such growth inhibition is not due to nonspecific cytotoxicity of STI 571 (*see* Moses et al., *supra*). The c-Kit-mediated inhibition by STI 571 of KSHV-infected DMVEC proliferation identifies STI 571 as a therapeutic modulator of KSHV-induced gene expression.

Additionally, as discussed herein, KSHV-infected DMVEC develop a spindle phenotype and exhibit transformed characteristics including disorganized growth, focus formation and anchorage-independent growth in semisolid agar. Following treatment of KSHV-infected DMVEC with STI 571 to inhibit endogenous c-Kit tyrosine kinase activity, focus formation is inhibited and an organized monolayer with distinct cell margins is reestablished (*Id*). Moreover, removal of STI 571 leads to regeneration of the transformed phenotype, even after exposure of cells to a 10 μM dose (*Id*). Uninfected DMVEC exhibit normal growth with an organized cobblestone phenotype when maintained at confluency, and exposure to STI 571 has effect on cell morphology or viability.

The ability to reverse KSHV-induced morphological transformation through specific inhibition of c-Kit activity further demonstrates a critical role for c-Kit signaling in KSHV-

induced transformation of endothelial cells and further supports a role for upregulation of c-Kit as a factor in KS tumorigenesis.

Likewise, modulators of the present novel validated KSHV-induced cellular gene expression are identified by the inventive screening assays.

Methods for Assessing the Efficacy of Modulators of either KSHV-induced Gene Expression or of Biological Activity Encoded thereby

Inventive modulators or compounds, whether antisense molecules or ribozymes, proteins and/or peptides, antibodies and/or antibody fragments or small molecules, that are identified either by one of the methods described herein or via techniques that are otherwise available in the art, may be further characterized in a variety of *in vitro*, *ex vivo* and *in vivo* animal model assay systems for their ability to modulate or inhibit KSHV-induced gene expression or biological activity. As discussed in further detail in the Examples provided below, particular inventive modulators of KSHV-induced gene expression are antisense inhibitors effective in reducing KSHV-induced cellular gene expression levels. Thus, the present invention describes, teaches and supports methods that permit the skilled artisan to assess the effect of candidate modulators and inhibitors.

For example, candidate modulators or inhibitors of KSHV-induced gene expression are tested by administration of such candidate modulators to cells that express KSHV-induced genes and gene products, such as KSHV-infected DMVEC in the inventive soft agar system. KSHV-infected mammalian cells may also be engineered to express a given KSHV-induced gene or recombinant reporter molecule introduced into such cells with a recombinant KSHV-inducible gene plasmid construct.

Effective modulators of KSHV-induced gene expression that are inhibitors will be effective in reducing the levels of KSHV-induced gene mRNA as determined, *e.g.*, by Northern blot or RT-PCR analysis. For a general description of these procedures, *see, e.g.*, Sambrook et al., *Molecular Cloning: A Laboratory Manual* Cold Spring Harbor Press (1989) and *Molecular Biotechnology: Principles and Applications of Recombinant DNA*, ASM Press (*ed.* Glick, B.R. and Pasternak, J.J. 1998) incorporated herein by reference. The effectiveness of a given candidate antisense molecule may be assessed by comparison with a control 'antisense' molecule (*e.g.*, a reverse complement control oligonucleotide, corresponding in orientation and size to the coding sequence complementary to the candidate antisense molecule) known to have no substantial effect on KSHV-induced gene expression when administered to a mammalian cell. Exemplary control molecules include KSHV-inducible gene sequence-specific reverse

complement oligonucleotides corresponding to one of the inventive antisense molecules described herein above, or to preferred representative thereof (*e.g.*, reverse complement control oligonucleotides for SEQ ID NOS:15-21 and SEQ ID NOS:31-33).

In alternate embodiments of the present invention, the effect of modulators and inhibitors of KSHV-induced gene expression on the rate of DNA synthesis after challenge with a radiation or chemotherapeutic agent may be assessed by, *e.g.*, the method of Young and Painter. *Hum. Genet.* (1989) *82*:113-117. Briefly, culture cells may be incubated in the presence of ^{14}C-thymidine prior to exposure to, *e.g.*, X-rays. Immediately after irradiation, cells are incubated for a short period prior to addition of ^{3}H-thymidine. Cells are washed, treated with perchloric acid and filtered (Whatman GF/C). The filters are rinsed with perchloric acid, 70% alcohol and then 100% ethanol; radioactivity is measured and the resulting ^{3}H/^{14}C ratios used to determine the rates of DNA synthesis.

Animal model systems. Modulators or inhibitors of KSHV-induced gene expression effective in modulating or reducing KSHV-induced cellular gene expression by one or more of the methods discussed above are further characterized *in vivo* for efficacy one or more available art-recognized animal model systems. Various animal model systems for study of cancer and genetic instability associated genes are disclosed in, for example, Donehower, L.A. *Cancer Surveys* (1997) *29*:329-352 incorporated herein by reference. In particular, various art-recognized animal model systems for testing PMO antisense oligonucleotide agents, including xenograft murine models are discussed Devi, *Current Opinion in Molecular Therapeutics*, 4:138-148, 2002, incorporated by reference herein.

Pharmaceutical Compositions

The antisense oligonucleotides and ribozymes of the present invention are synthesized by any method known in the art for ribonucleic or deoxyribonucleic nucleotides. For example, the oligonucleotides are prepared using solid-phase synthesis such as in an Applied Biosystems 380B DNA synthesizer. Final purity of the oligonucleotides is determined as is known in the art.

The antisense oligonucleotides identified using the methods of the invention modulate cancer cell proliferation, including anchorage-independent proliferation, and also modulate KSHV-mediated phenotypic changes, including spindle formation.

Therefore, pharmaceutical compositions and methods are provided for interfering with cell proliferation, preferably cancer or tumor cell proliferation, comprising contacting tissues or cells with one or more of antisense oligonucleotides identified using the methods of the

invention. Preferably, an antisense oligonucleotide having one of SEQ ID NOS:15-21 and SEQ ID NOS:31-33 is administered. Preferably, the antisense oligonucleotide is a PMO antisense oligomer (PMO).

The methods and compositions may also be used to treat other KSHV-associated proliferative disorders including sarcomas, and KSHV-related neoangiogenesis (neovascularization).

The invention provides pharmaceutical compositions of antisense oligonucleotides and ribozymes complementary to validated KSHV-induced cellular gene mRNA gene sequences, corresponding to SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29 as active ingredients for therapeutic application. These compositions can also be used in the methods of the present invention. Where required the compounds are nuclease resistant. In general the pharmaceutical composition for modulating KSHV-mediated cellular proliferation or phenotype in a mammal includes an effective amount of at least one antisense oligonucleotide as described above needed for the practice of the invention, or a fragment thereof shown to have the same effect, and a pharmaceutically physiologically acceptable carrier or diluent.

Particular embodiments provide a method for reducing KSHV-mediated cellular proliferation and/or phenotypic differentiation in a subject comprising administering an amount of an antisense oligonucleotide of the invention effective to reduce said KSHV-mediated cellular proliferation and/or phenotypic differentiation. Preferably the antisense oligomer is based on one of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29. More preferably the antisense oligonucleotide is one of SEQ ID NOS:15-21 and SEQ ID NOS:31-33.

The pharmaceutical composition for inhibiting tumorigenicity of neoplastic cells in a mammal consists of an effective amount of at least one active ingredient selected from antisense oligonucleotides complementary to the KSHV-induced cellular gene mRNA, including the entire KSHV-induced gene mRNA or having shorter sequences as set forth in SEQ ID NOS:15-21 and SEQ ID NOS:31-33, and a pharmaceutically acceptable carrier or diluent. Combinations of the active ingredients are contemplated and encompassed within the scope of the invention.

The compositions can be administered orally, subcutaneously or parenterally including intravenous, intraarterial, intramuscular, intraperitoneally, and intranasal administration as well as intrathecal and infusion techniques as required by the malignant cells being treated. For delivery within the CNS intrathecal delivery can be used with for example an Ommaya reservoir or other methods known in the art. The pharmaceutically acceptable carriers, diluents, adjuvants and vehicles as well as implant carriers generally refer to inert, non-toxic solid or liquid fillers, diluents or encapsulating material not reacting with the active ingredients of the invention.

Cationic lipids may also be included in the composition to facilitate oligonucleotide uptake. Implants of the compounds are also useful. In general, the pharmaceutical compositions are sterile.

In the method of the present invention, KSHV-related proliferating cells, including neoplastic cells are contacted with a growth-inhibiting amount of the bioactive antisense oligonucleotide for the KSHV-induced cellular gene mRNA or a fragment thereof shown to have substantially the same effect. In an embodiment, the mammal to be treated is human but other mammalian species can be treated in veterinary applications.

Bioactivity, relating to a particular oligonucleotide modulator, refers to biological activity in the cell when the oligonucleotide modulator is delivered directly to the cell and/or is expressed by an appropriate promotor and active when delivered to the cell in a vector as described below. Nuclease resistance of particular modulators is provided by any method known in the art that does not substantially interfere with biological activity as described herein.

Significantly, PMO chemistry is not RNase H competent (*discussed in* Devi, *Current Opinion in Molecular Therapeutics*, 4:138-148, 2002).

"Contacting the cell" refers to methods of exposing, delivery to, or 'loading' of a cell of antisense oligonucleotides whether directly or by viral or non-viral vectors, and where the antisense oligonucleotide is bioactive upon delivery. The method of delivery will be chosen for the particular cancer being treated. Parameters that affect delivery can include the cell type affected and tumor location as is known in the medical art.

The treatment generally has a length proportional to the length of the disease process and drug effectiveness and the patient species being treated. It is noted that humans are treated generally longer than the Examples exemplified herein, which treatment has a length proportional to the length of the disease process and drug effectiveness. The doses may be single doses or multiple doses as determined by the medical practitioners and treatment courses will be repeated as necessary until diminution of the disease is achieved. Optimal dosing schedules may be calculated using measurements of drug accumulation in the body. Practitioners of ordinary skill in the art can readily determine optimum dosages, dosing methodologies, and repetition rates. Optimum dosages may vary depending on the relative potency of the antisense oligonucleotide, and can generally be determined based on values in *in vitro* and *in vivo* animal studies and clinical trials. Variations in the embodiments used may also be utilized. The amount must be effective to achieve improvement including but not limited to decreased tumor growth, or tumor size reduction, or to improved survival rate or length or

decreased drug resistance or other indicators as are selected as appropriate measures by those skilled in the art.

Although particular inventive antisense oligonucleotides may not completely abolish tumor cell growth, or KSHV-induced proliferation or differentiation *in vitro*, as exemplified herein, these antisense compounds are nonetheless clinically useful where they inhibit KSHV-related tumor growth enough to allow complementary treatments, such as chemotherapy or radiation therapy, to be effective or more effective. The pharmaceutical compositions of the present invention therefore are administered singly or in combination with other drugs, such as cytotoxic agents, immunotoxins, alkylating agents, anti-metabolites, antitumor antibiotics and other anti-cancer drugs and treatment modalities that are known in the art.

Cocktails of antisense inhibitors directed against several KSHV-induced gene sequences are contemplated and within the scope of the present invention.

The composition is administered and dosed in accordance with good medical practice taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, and other factors known to medical practitioners. The "effective amount" for growth inhibition is thus determined by such considerations as are known in the art. The pharmaceutical composition may contain more than one embodiment or modulator of the present invention.

The nucleotide sequences of the present invention can be delivered either directly or with viral or non-viral vectors. When delivered directly the sequences are generally rendered nuclease resistant. Alternatively, the sequences can be incorporated into expression cassettes or constructs such that the sequence is expressed in the cell. Generally, the construct contains the proper regulatory sequence or promoter to allow the sequence to be expressed in the targeted cell.

Once the oligonucleotide sequences are ready for delivery, they can be introduced into cells as is known in the art (*see, e.g.*, Devi, *Current Opinion in Molecular Therapeutics*, 4:138-148, 2002). Transfection, electroporation, fusion, liposomes, colloidal polymeric particles and viral vectors as well as other means known in the art may be used to deliver the oligonucleotide sequences to the cell. The method selected will depend at least on the cells to be treated and the location of the cells and will be known to those skilled in the art. Localization can be achieved by liposomes, having specific markers on the surface for directing the liposome, by having injection directly into the tissue containing the target cells, by having depot associated in spatial proximity with the target cells, specific receptor mediated uptake, viral vectors, or the like.

Administration and clinical dosing of PMO antisense therapeutic agents is discussed, for example, in Devi, *supra*, and in Arora et al. *Journal of Pharmaceutical Sciences*, 91:1009-1018, 2001, both incorporated by reference herein.

The present invention provides vectors comprising an expression control sequence operatively linked to the oligonucleotide sequences of the invention. The present invention further provides host cells, selected from suitable eukaryotic and prokaryotic cells, which are transformed with these vectors as necessary. Such transformed cells allow the study of the function and the regulation of malignancy and the treatment therapy of the present invention.

Vectors are known or can be constructed by those skilled in the art and should contain all expression elements necessary to achieve the desired transcription of the sequences. Other beneficial characteristics can also be contained within the vectors such as mechanisms for recovery of the oligonucleotides in a different form. Phagemids are a specific example of such beneficial vectors because they can be used either as plasmids or as bacteriophage vectors. Examples of other vectors include viruses such as bacteriophages, baculoviruses and retroviruses, DNA viruses, liposomes and other recombination vectors. The vectors can also contain elements for use in either prokaryotic or eukaryotic host systems. One of ordinary skill in the art will know which host systems are compatible with a particular vector.

The vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995), Vega et al., Gene Targeting, CRC Press, Ann Arbor, Mich. (1995), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass. (1988) and Gilboa et al., *BioTechniques* (1986) *4*:504-512 and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors.

Recombinant methods known in the art can also be used to achieve the antisense inhibition of a validated target nucleic acid. For example, vectors containing antisense nucleic acids can be employed to express an antisense message to reduce the expression of the validated target nucleic acid and therefore its activity.

The present invention also provides a method of evaluating if a compound inhibits transcription or translation of an KSHV-induced cellular gene sequence, and thereby modulates (*i.e.*, reduces) cell proliferation or phenotypic differentiation, comprising transfecting a cell with an expression vector comprising a nucleic acid sequence encoding a KSHV-induced cellular

gene sequence, the necessary elements for the transcription or translation of the nucleic acid; administering a test compound; and comparing the level of expression of the KSHV-induced cellular gene sequence with the level obtained with a control in the absence of the test compound. Alternatively, as is shown in the Examples herein, such an expression vector is not required, and test compounds are administered to KSHV-infected cells, such as KSHV-infected DMVEC.

The present invention provides detectably labeled oligonucleotides for imaging KSHV-induced cellular gene sequences (polynucleotides) within a cell. Such oligonucleotides are useful for determining if gene amplification has occurred, for assaying the expression levels in a cell or tissue using, for example, *in situ* hybridization as is known in the art, and for diagnostic and/or prognostic purposes.

Diagnostic and/or Prognostic Assays for KSHV-related Cancer

The present invention provides for diagnostic and/or prognostic cancer assays based on differential measurement of validated KSHV-induced gene expression. Preferred validated KSHV-induced gene sequences are represented herein by SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29.

Typically, such assays involve obtaining a tissue sample from a test tissue, performing an assay to measure expression of at least one validated KSHV-induced gene sequence (*e.g.*, mRNA or protein encoded thereby) derived from the tissue sample, relative to a control sample, and making a diagnosis or prognosis based thereon.

In particular embodiments the present inventive oligomers, such as those based on SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29, or preferably SEQ ID NOS:15-21 and SEQ ID NOS:31-33, or arrays thereof, as well as a kit based thereon are useful for the diagnosis and/or prognosis of cancer and/or other KSHV-related cell proliferative disorders.

The present invention moreover relates to a method for manufacturing a diagnostic agent and/or therapeutic agent for the diagnosis and/or therapy of KSHV-related diseases, the diagnostic agent and/or therapeutic agent being characterized in that at least one inventive modulator of KSHV-induced gene expression is used for manufacturing it, possibly together with suitable additives and ancillary agents.

Diagnostic kits are also contemplated, comprising at least one primer and/or probe specific for a validated KSHV-induced cellular gene sequence according to the present invention, possibly together with suitable additives and ancillary agents.

While the present invention has been described with specificity in accordance with certain of its preferred embodiments, the following examples serve only to illustrate the invention and are not intended to limit the invention.

EXAMPLE 1

(KSHV-infected DMVECs are a valid model system for *in vivo* tumorogenesis)

Soft Agar Cell Growth Systems. The soft agar assay system is an art-recognized *in vitro* cell growth/differentiation system to model *in vivo* cancer. Particularly, out of a host of exemplary references, see: Tomkowicz, K et al., *DNA Cell Biol.* 21:151, 2002 (use of soft agar assays system to demonstrate transformation with KSHV kaposin protein); Saucier et al., *Oncogene* 21:1800, 2002 (use of soft agar assays system to demonstrate transformation with Met RTK protein); and *see also* Chernicky, CL, *Mol. Pathol.* 55:102, 2002 (use of inhibition of colony formation in soft agar as validation for siRNS inhibition of a tumor growth factor).

KSHV-infected DMVEC. DMVECs were used as an *in vitro* model for examining cancerous transformation and viral replication, based, *inter alia*, on that fact that neoplastic cells in KS tumors are predominantly of vascular origin, whereas KSHV is primarily found in cells of endothelial origin. Specifically, a previously described DMVEC system (Moses et al., *J. Virol.* 73:6892-6902, 1999) was used for studying infection and transformation by KSHV. Briefly, DMVEC's were immortalized with the E6/E7 genes of human papillomavirus (HPV)-16 prior to infection with KSHV. While transformation with HPV-E6 and HPV-E7 immortalizes DMVEC, they do not develop the KS-typical spindle shape (Staskus, K. A., et al., *J Virol.* 71:715-9, 1997) unless infected with KSHV. KSHV was obtained from the supernatant of KSHV-infected B-cell lines (*e.g.*, TPA-stimulated BCBL-1 cells). Infection was verified by DNA PCR for amplification of the KS330 BamH1 fragment of the ORF 26 gene, and RT-PCR for the spliced mRNA from the ORF29 gene. The percentage of latently infected cells was determined by immunofluorescent staining for LANA/ORF73. Lytic induction was evaluated with antibodies against an early lytic protein ORF59/PF-8 and a late lytic glycoprotein ORF K8.1A/B. DMVEC were used for experiments when 90% of cells expressed ORF73. In the absence of chemical induction, 2-5% of infected cells expressed ORF59 with approximately 10% of the ORF59-positive cells expressing K8.1A/B. Lytic replication can be induced, however, using phorbol esters such as phorbol-112-myristate-13 acetate (PMA) providing the ability to look for host genes involved in the lytic cycle as well.

Figures 1A, B and C show data from experiments performed to illustrate three hallmarks of the KSHV-DMVEC model system that support its art-recognized utility for mimicking the *in vivo* system.

First, Figure 1A shows that immortalized DMVEC cells grow with a characteristic cobblestone morphology in the absence of KSHV infection but change to a *spindle cell* morphology one (central-panel) to four weeks (rightmost-panel) following infection with KSHV. Specifically, Figure 1A shows dermal microvascular endothelial cells (DMVECs) that were uninfected ("Mock") (left-most panel), 1-week post-infection (central panel), or 4-weeks post-infection (right-most panel). The beginning of characteristic *spindle cell* formation in DMVEC cells was observed 1-week post-infection with KSHV, and substantially progressed through 4 weeks post-infection.

Figure 1B shows a second feature of the KSHV-DMVEC model system that mimics the *in vivo* situation; namely, that KSHV enters the lytic replication cycle spontaneously in only approximately 2% of the cells (compare left-most and central panels of Figure 1B). This ratio, as described above, was visualized by immunofluorescence with antibodies that recognize the products of viral genes expressed during latency (ORF 73, LANA-1) (left-most panel) or viral proteins that are only expressed upon entering the lytic phase (ORF 59) (central panel). Lytic replication can be, and was induced, however, using phorbol esters such as PMA providing the ability to look for host genes involved in the lytic cycle as well (right-most panel). Specifically, Figure 1B shows fluorescent staining of latent KSHV-infected DMVEC cells ("ORF7," left-most panel), fluorescent staining of lytic KSHV infected DMVEC cells ("B-ORF59," central panel), and fluorescent staining of lytic KSHV-infected DMVEC cells enhanced with PMA ("ORF59+PMA," right-most panel). Phorbol-112-myristate-13 acetate (PMA) was purchased from Calbiochem (San Diego, CA).

Third, Figure 1C shows that while immortalized DMVECs are unable to form foci or grow in soft agar in the absence of KSHV infection, they exhibit hallmarks of transformation following KSHV infection; namely, loss of contact inhibition, and acquisition of anchorage-independent growth. Specifically, Figure 1C shows the beginning of foci formation in KSHV-infected DMVEC observed at 1-week post infection ("KSHV 1 week," left-most panel), progression of foci formation observed at 4-weeks post infection ("KSHV 4 weeks," central panel), and KSHV-infected DMVECs observed growing in soft agar as a result of the acquisition of anchorage-independent growth ("KSHV Agar," right-most panel).

These phenotype changes, illustrated by the experimental data of Figures 1A, B and C, formed the basis for the primary biological assays used herein to validate regulated cellular genes and/or gene products as therapeutic targets.

EXAMPLE 2

(Nucleic acid microarray technology was used for gene expression profiling of KSHV-infected dermal microvascular endothelial cells (DMVEC) to identify cellular genes whose expression is regulated by KSHV)

Nucleic Acid Microarray Data Analysis. Altered expression of cellular genes frequently represents the ultimate cause of tumor formation. In the case of virally-induced tumors, viral genes modulate the host cell gene expression program that is in turn responsible for the transformed phenotype. Cellular genes involved in the transformed phenotype caused by latent infection with KSHV were identified by using DNA microarrays to examine the differential gene expression profiles of primary dermal microvascular endothelial cells (DMVEC) before and after KSHV-infection.

For RNA isolation and fluorescent labeling, two RNA probe samples from DMVEC cells, independently infected with KSHV, and two independent uninfected RNA probe samples were prepared. Briefly, experiments were performed on cells shortly after spread of infection to the majority of cells and development of spindle cells. Specifically, RNA was routinely isolated approximately 4-6 weeks post-infection, after initial infection when >90% of the cells were LANA positive and showed spindle cell phenotype. RNA was isolated from T75 flasks containing approximately 5×10^6 cells using the RNeasy™ RNA isolation kit (QIAGEN Inc., Valencia, CA). After DNase treatment and another round of RNeasy purification, labeled cDNA was prepared as described previously (*see* Salunga et al., *In* M. Schena (ed.), DNA microarrays. A practical approach; Oxford Press, Oxford, United Kingdom, 1999; *and see* Simmen et al., *Proc. Natl. Acad. Sci. USA* 98:7140-7145, 2001). Briefly, double-stranded cDNA was selectively synthesized from the RNA samples. Biotin-labeled cRNA was produced from the cDNA by in vitro transcription (IVT) using methods well known in the art.

For expression profile screening, the biotin labled cRNA probe preparations were fragmented and hybridized to Affymetrix (Santa Clara, CA) U133A and U133B arrays or to U95A arrays (Affymetrix U133A, U133B and U95A GeneChip® arrays). The Human Genome U133 (HG-U133) set, consists of two GeneChip® arrays, and contains almost 45,000 probe sets representing more than 39,000 transcripts derived from approximately 33,000 well-substantiated

human genes (Affymetrix technical information). The set design uses sequences selected from GenBank®, dbEST, and RefSeq (*Id*).

The Affymetrix GeneChip® platform was chosen for these studies as it is the industry leader in terms of array content, platform stability and data quality. Images of the arrays were analyzed using the Affymetrix microarray analysis suite software, MAS. This software package is used for converting images to raw numerical data, and direct comparisons between control and experimental samples. When making such comparisons, MAS provides robust statistical algorithms for determining changes in expression between the two samples, along with p-values and confidence limits on such changes. For each probe set, MAS records whether there was no change, increased expression or decreased expression.

To determine if the number of gene expression changes in common between two or more experiments is significant, we compare the number of genes in such lists to the number expected if the experiments were independent. In the present KSHV experiments, there are approximately 10-fold more gene changes in common between infections than predicted for independent experiments.

Each of the DMVEC infected/uninfected sample comparisons resulted in approximately 480 probe sets with increased expression, with 316 probe sets that showed increased expression in both infections. There were 390 probes sets that showed decreased expression in both, out of approximately 600 probe sets that were down in the individual experiments. Increased or decreased expression was based on 'calls' from MAS software which typically corresponds to about a two-fold change. The 706 probes sets identified with significant changes in expression correspond to 580 unique gene sequences.

Representative microarray expression data. TABLE 1 shows expression data obtained according to the present invention for the RDC1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT, IFACTOR, LMO2, MFAP3, LOX, NOV and ANGTPL2 gene sequences using Affymetrix U133 and U95 arrays as indicated. Expression is presented as "fold-increase" in signal for two to four independent infected/mock infected comparisons, as described herein above.

TABLE 1. U133 and U95A microarray expression data for particular KSHV-induced gene sequences.

GENE	ARRAY	Affymetrix Probe Set	FOLD INCREASE; I1219 x M1219	FOLD INCREASE; I0109 x M0109

GENE	ARRAY	Affymetrix Probe Set	FOLD INCREASE; I1219 x M1219	FOLD INCREASE; I0109 x M0109
RDC-1	UI33A	212977_at	34	87
	U95A	34288_at	37.9	36.1
IGFBP2	UI33A	202718_at	2.7	1.8
	U95A	40422_at	2.3	3.5
FLJ14103	UI33A	219652_s_at	30.2	44.7
	UI33A	222911_s_at	3.8	4.7
KIAA0367	U133A	212805_at	2.4	2.6
	U133A	212806_at	3.2	2.6
	U95A	33442_at	3.3	3.2
Neuritin	n/a	n/a	n/a	n/a
INSR	U133A	213792_s_at	2.6	2.7
	U133B	227432_s_at	2.5	3.4
	U95A	1572_s_at	3.6	11.4
KIT	U133A	205051_s_at	34	20.9
	U95A	1888_s_at	~10.8	~30.1
IFACTOR	UI33A	203854_at	21.6	39.4
LMO2	UI33A	204249_s_at	2.2	2.8
MFAP3	UI33A	213123_at	2.5	2.7
	UI33A	214588_s_at	10.9	4.4
	U95A	35217_at	3.4	4.5
LOX	U133A	215446_s_at	1.62	3.48
	U133A	213640_s_at	1.07	2.3
	U133A	204298_s_at	1.32	3.48
NOV	U133A	214321_at	5.66	8
	U133A	204501_at	2.83	5.28
ANGPTL2	U133A	213004_at	1.52	3.03
	U133A	213001_at	1.74	3.48

Functional grouping of identified gene sequences. Figure 2 shows a placement of the genes identified as having statistically significant altered expression in KSHV-infected (latent) DMVEC into functional groups, based on information available in the art.

EXAMPLE 3

(Target validation; genes necessary for virally-induced morphological changes in KSHV-infected DMVEC were identified using antisense PMOs)

Antisense Phosphorodiamidate Morpholino Oligomers (PMOs). PMOs (see, *e.g.*, Summerton, et al., *Antisense Nucleic Acid Drug Dev.* 7:63-70, 1997; and Summerton & Weller,

Antisense Nucleic Acid Drug Dev. 7:187-95, 1997) are a class of antisense drugs developed for treating various diseases, including cancer. For example, Arora et al. (*J. Pharmaceutical Sciences* 91:1009-1018, 2002) demonstrated that oral administration of *c-myc*-specific and CYP3A2-specific PMOs inhibited *c-myc* and CYP3A2 gene expression, respectively, in rat liver by an antisense mechanism of action. Likewise, Devi G.R. (*Current Opinion in Molecular Therapeutics* 4:138-148, 2002) discusses treatment of prostate cancer with various PMO therapeutic agents).

PMOs were designed and used, according to the present invention to silence genes identified as being consistently up-regulated in KSHV-infected DMVEC. PMOs do not activate RNAse H, and inhibit translation by steric hindrance at the ribosome binding site (Ghosh, et al. *Methods in Enzymology* 313:135-143, 2000). Typically, it is preferable and sufficient to target the region of the start codon to block translation, but, as discussed herein above, other mRNA regions, both coding and non-coding can be effectively targeted according to the present invention.

Antisense Gene Silencing using PMOs. Genes identified as being consistently up-regulated in KSHV-infected DMVEC in the above described nucleic acid microarray/gene expression profiling experiments were further analyzed to identify those necessary for virally-induced cell morphology changes. Silencing of such genes precluded progression into the transformed phenotype when silencing occurred prior to transformation, or induced reversion to the normal state when silencing occurred after induction of the transformed state (*see* TABLE 2 below).

Therefore, the present invention provides for particular validated cellular gene targets, and for respective therapeutic methods and compositions for blocking virally-induced morphological changes and treating or preventing cancer.

Introduction of antisense PMO into KSHV-infected DMVEC. Antisense PMO molecules, for delivery purposes, are typically converted to a paired duplex together with a *partially* complementary cDNA oligonucleotide in the weakly basic delivery reagent ethoxylated polyethylenimine (EPEI) (Summerton, *supra*). The anionic complex binds to the cell surface, is taken up by endocytosis and eventually released into the cytosol. A protocol for optimum uptake of antisense PMO in immortalized DMVEC was developed using a modification of the EPEI method. Briefly, uninfected, immortalized DMVECs were incubated for 3 hours at 37°C with 0.6 nmol/well FITC-PMO complexed with EPEI according to the manufacturer's instructions (Genetools, LLC, One Summerton Way, Philomath, OR 97370) (*e.g.*, 1.25 nMol oligomer with 2.5 µl EPEI reagent per 35 mm dish, allowing for sufficient antisense uptake

without non-specific EPEI-induced toxicity). The PMOs were labeled with FITC to allow for monitoring of loading efficiency by fluorescence microscopy.

Cellular distribution of introduced FITC-labeled POM antisense molecules. Figure 3A (lower-right panel "D") shows a representative fluorescent image of FITC-labeled c-Kit PMO antisense uptake. Specifically, the c Kit antisense PMO molecules were initially concentrated in intracellular vesicles (endosomes) at 3 hours in about 70% of the cells, and distributed within the cytoplasm at 66 hours. By contrast, no uptake was observed for control FITC-labeled proteins such as antibodies. Significantly, PMO oligomers were distributed within the entire cytoplasm and nuclei of treated cells at 66 hours (see Figure 3A, lower-right panel "D").

Therefore, the introduced PMO antisense oligomers were determined to be stable over substantial time periods in DMVEC. Significantly, stable staining (FITC) was observed for up to 10 days without any toxic effects. Moreover, the PMO oligomers were readily taken up by DMVEC and distributed within the cytosol.

Proof of principal for target validation; silencing of c-Kit gene expression. The efficacy of the PMO antisense strategy for gene expression silencing in the above-described KSHV-infected DMVEC system was demonstrated using a specific FITC-labeled PMO targeting the start codon of c-Kit (5'-CGCCTCTCATCGCGGTAGCTGCG-3'; SEQ ID NO:21), a protein previously shown by applicants to induce focus formation in KSHV-infected DMVEC (Moses, et al., *J. Virology* 76:8383-99, 2002.).

Specifically, DMVEC were infected with KSHV, plated in 35mm dishes and allowed to grow to about 90% confluence. For treatment, KSHV-infected cells were treated with the anti-c-Kit PMO-antisense oligomer-EPEI delivery reagent complex and incubated for 3 hours at 37°C in serum-free medium to allow for oligomer uptake. A titration experiment testing a range of different oligomer/EPEI volumes was used to determine that loading 1.25 nmol oligomer with 2.5 µl EPEI reagent per 35 mm dish allowed efficient antisense uptake without non-specific EPEI-induced toxicity. Control (mock-treated) DMVEC cultures were loaded with EPEI reagent and sterile water or sterile water alone. Upon removal of the oligomer-EPEI solution, cell monolayers were rinsed in serum-free medium fed with complete medium and examined daily for one week by phase microscopy for evidence of phenotypic change.

Figure 3A (panels "A," "B" and "C") shows that treatment with c-Kit PMO antisense (SEQ ID NO:21) resulted in restoring contact-inhibited growth of KSHV-infected DMVECs. Specfically, Figure 3A (upper-left panel "A") shows that during the week of post-loading

culture, untreated KSHV-infected DMVECs approached confluence and were maintained in a post-confluent state. Such untreated DMVEC exhibited loss of contact inhibition and the capacity to grow in disorganized, multi-layered foci that were evident by day 6 post-loading (Figure 3A, upper-left panel "A"). Likewise, cells cultured with 2.5 µl EPEI alone (treatment control) showed similar focus formation (Figure 3A, upper-right panel "B"). Significantly, cells loaded with 1.25 nmol of the c-Kit antisense PMO oligomer and 2.5 µl EPEI (treated cells) did not develop foci, and maintained a quiescent contact-inhibited monolayer (Figure 3A, lower-left panel "C").

As described above, a direct role of c-Kit over-expression in DMVEC morphologic alteration has been previously demonstrated (Moses, et al., *J. Virology* 76:8383-99, 2002.). Therefore, the blockade of spindle cell, and foci formation observed herein confirms that the c-Kit antisense PMO oligomer was substantially effective in inhibiting c-Kit expression/function.

Figure 3B shows evidence that despite expression in some cells of c-kit protein, the cell cultures treated with c-Kit antisense PMO oligomer (SEQ ID NO:21) did not progress to spindle cell and foci formation (*see* phase contrast images of Figure 3A, lower-left panel "C").

Validation of KSHV-induced gene sequences. TABLE 2 shows the validation results for thirteen induced genes identified in the experiments of EXAMPLE 2 herein above. For seven of the induced genes, suppression by sequence-specific PMO antisense oligonucleotides led to inhibitory effects (either full or intermediate inhibition) on KSHV-induced spindle cell formation in DMVEC, including two *novel* genes and an orphan G-protein coupled receptor. Silencing of seven of the genes (RDC-1 (GPCR RDC1), IGFBP2 (insulin-like growth factor binding protein 2), FLJ14103 (hypothetical protein FLJ14103), Neuritin, KIT (c-KIT), LOX (lysyl oxidase preprotein) and Nov (nov precursor)) resulted in fully reversed spindle cell formation, while intermediate inhibitory effects were seen for three of the genes (KIAA0367 (KIAA0367 protein), INSR (Insulin receptor) and ANGPTL2 (angiopoietin-like 2 precursor)). The specific PMO antisense oligomers used in these experiments for silencing the KSHV-induced gene sequences are also shown in TABLE 4, along with corresponding SEQ ID NOS.

TABLE 2. Validated Gene Targets; suppression (silencing) of particular KSHV-induced genes prevented or significantly inhibited KSHV-induced spindle cell formation.

GENE	PMO Antisense Sequence (5' to 3')		Extent of PMO-induced Inhibition of Spindle Cell Formation
RDC-1	GAAGAGATGCAGATCCATCGTTCTG	(SEQ ID NO:15)	full
IGFBP2	GGCAGCCCACTCTCTCGGCAGCATG	(SEQ ID NO:16)	full
FLJ14103	GGCTCCATCTTGGGCTCTGGGCTCC	(SEQ ID NO:17)	full
KIAA0367	GTCAGTTTACTCATGTCATCTATTG	(SEQ ID NO:18)	intermediate
Neuritin	TTAACTCCCATCCTACGTTTAGTCA	(SEQ ID NO:19)	full
INSR	GGGTCTCCTCGGATCAGGCGCG	(SEQ ID NO:20)	intermediate
KIT	CGCCTCTCATCGCGGTAGCTGCG	(SEQ ID NO:21)	full
IFACTOR	AGCTTCATGTTGGAGGTGTTCG	(SEQ ID NO:22)	none
LMO2	GCCGAGGACATTGGGGAGGGAGGCG	(SEQ ID NO:23)	none
MFAP3	TGAATAAGCAACAATGTAGCTTCAT	(SEQ ID NO:24)	none
LOX	GGAGCACGGTCCAGGCGAAGCGCAT	(SEQ ID NO:31)	full
NOV	AGCTCGTGCTCTGCACACTCTGCAT	(SEQ ID NO:32)	full
ANGPTL2	AGCATGTCACGCACAGTGGCCTCAT	(SEQ ID NO:33)	intermediate

TABLE 3 summarizes GenBank mRNA and EST accession numbers for particular KSHV-induced genes, including for the ten validated gene sequences listed in TABLE 2. Gene names, Unigene clusters (from build #153), and GenBank accession numbers for these validated sequences are as assigned by the National Center for Biotechnology Information (NCBI), and are incorporated by reference herein, including all splice and allelic variants of these mRNA sequences.

TABLE 3. GenBank accession numbers for particular KSHV-induced genes, including for the RDC1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT, LOX, NOV and ANGPTL2 gene sequences validated herein.

GENE	Unigene Cluster	Accession Numbers; mRNAs	Accession Numbers; ESTs
RDC-1	Hs.23016	BI460261	BI767134, BM921366, BM925428, BM458484, R27256, AI954295, AA205847, AA197246, AI633054
IGFBP2	Hs.162	BC004312, M35410, NM_000597, BC009902, BC012769, X16302	BE382548, BM564454, BM928278, BM545072, BI830342, BE382760, BE313151, BF981949, BM548711
FLJ14103	Hs.98321	AK024165	BI818834, T75260, R38645, AI796127, AI095506, W61099, W63748, AI554899, AA689489, AI631711

GENE	Unigene Cluster	Accession Numbers; mRNAs	Accession Numbers; ESTs
KIAA0367	Hs.23311	AB002365, BC022571, AL834213	BI457935, BI552977, BG706827, R21961, R25052, R45391, H05195, H05155, R25051, R45390
Neuritin	Hs.103291	AF136631, BC002683, NM_016588, AJ420483, AK093824	BI918095, BI548839, BI602117, BI915704, BE897829 , BI824717, BG714127, BQ231718, BF970432, BF966251
INSR	Hs.89695	X02160, M10051, NM_000208	AA860814, AA486513, AA485908, H03917, AI738814, AA613904, AA632501, AA632558, AA632596, W52906
KIT	Hs.81665	NM_000222, X06182	BF966487, AI567686, AI567693, AI674108, AI308810, N20798 , AA873164 , AI017093, H10570 , R35401
IFACTOR	Hs.36602	NM_000204, BC020718, J02770	BM924043, BF132103, BG435910 ,BG431258, BG568130, BG401433, BG426851, BG566266, BI761434, BQ277394
LMO2	Hs.184585	NM_005574, BC034041 , X61118 , AF257211	BI764252, BM808939, BG715963, BG505616, R60732, AI337730, AW005586, AI687026, H10900, AI979150
MFAP3	Hs.28785	AL049404, NM_005927, BC026244, AK000358	BG531421, AI684093, AI933971, H60952, H61526, H99277, AI874390, R95175, AI452602, R13620
LOX	Hs.102267	AF039291.1, NM_002317.3, M94054.1, S78694.1, S45875.1	N26939.1, H99075.1, AW005592.1, AI761085.1, AA599304.1, AI075382.1, AI022363.1, AI075456.1, AI335739.1, AA099452.1
NOV	Hs.235935	NM_002514.2, X96584.1, BC015028.1, AY082381.1	H15316.1, R25930.1, AI920781.1, AA081850.1, AI055954.1, AA604355.1, R41819.1, AI923336.1, H29804.1, H29805.1
ANGPTL2	Hs.8025	NM_012098.1, AF125175.1, BC012368.1, AK075026.1, AK074726.1, AF007150.1	AA255567.1, AA617726.1, AI677659.1, AI934310.1, T77327.1, R38293.1, R51659.1, R51569.1, R47836.1, R51427.1

Inhibition of KSHV-induced cellular proliferation by PMO antisense inhibition. KSHV-infected DMVEC, as described above under EXAMPLE 1, lose the characteristic contact-inhibition displayed by DMVEC, and proliferate in response to virally-induced regulatory signals. Therefore, in addition to the inhibition/reversion of spindle-cell formation, further validation of KSHV-related cellular gene targets was achieved by determining whether silencing

of particular KSHV-induced gene sequences resulted in the inhibition of KSHV-induced DMVEC proliferation. As shown below, PMO-mediated gene silencing resulted in the inhibition of KSHV-induced DMVEC proliferation, and these results correlated with the ability of the respective PMOs to inhibit spindle cell formation (phenotypic inhibition).

Proliferation assays, and loading of cells with PMOs. Proliferation of KSHV-infected DMVEC was quantified using an XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5- carboxanilide, disodium salt)-based assay. KSHV-infected cells were added to Primaria 96-well trays (Becton Dickinson) at 1×10^4 or 5×10^4 cells/well. XTT (Roche, Molecular Biochemicals, Indianapolis, IN) was added 48 hours later according to the manufacturer's instructions. Absorbance was read after 4 to 6 hours on a microplate reader.

Briefly, cells were plated in 96-well trays at a density approaching confluence (5×10^4 cells per well) in 100 µl of complete medium. PMOs were loaded the following day in a total of 100 µl (0.5 µl PMO, 0.5 µl EPEI, 49 µl H_2O and 50 µl serum free medium) with reagent mixing as described by the manufacturer (GeneTools). Controls included a FITC PMO control oligonucleotide, EPEI only or H_2O only. Each variable was performed in quadruplicate. Fresh complete medium was replaced 4 hours after loading. Cells were cultured for 4 days to allow for multi-layered cell growth post-confluence in the absence of any growth inhibition. XTT was added on day 4 of culture and the absorbance read 4 hrs later on a microplate reader. Cell proliferation (growth) values are given as percentage inhibition values, relative to cells without PMO, which are adjusted to 100%.

TABLE 4 (center column) shows the extent of inhibition of KSHV-induced proliferation by specific PMO antisense inhibition of target genes (left column) as measured by XTT cellular proliferation assays. Corresponding phenotype inhibition values (extent of inhibition of spindle cell formation) are also shown (right column), based on experiments as outlined in EXAMPLE 2, herein above.

TABLE 4. Target gene-specific PMO antisense treatment; comparison between the extent of inhibition of KSHV-induced proliferation, and corresponding phenotype inhibition values.

GENE	Growth Inhibition (% of control)	Phenotype Inhibition (inhibition of spindle cell formation)
IGFBP2	55%	Full
c-Kit	50%	Full
RDC-1	43%	Full
Neuritin	29%	Full
KIAA0367	28%	Intermediate

GENE	Growth Inhibition (% of control)	Phenotype Inhibition (inhibition of spindle cell formation)
INSR	26%	Intermediate
I-Factor	12%	None
MFAP	11%	None
Osteopontin	4%	None
LOX		Full
NOV		Full
ANGPTL2		Intermediate

Consistent with the above-described results for inhibition of spindle formation, PMO antisense oligonucleotide inhibition (silencing) of the validated targets, including c-Kit, RDC-1, IGFB-2, Neurtitin, KIAA0367 and INSR resulted in substantial inhibition of KSHV-induced cellular proliferation.

By contrast, silencing of other KSHV-induced gene sequences, such as MFAP, I-Factor and Osteopontin resulted in relatively little or no significant inhibition of KSHV-induced cellular proliferation. Significantly, these results are consistent with PMO antisense results disclosed herein above, which excluded these KSHV-induced gene sequences from the validated target pool.

To further support and illustrate the correspondence between the extent of inhibition of KSHV-induced proliferation and corresponding phenotype inhibition values (full inhibition, intermediate inhibition and no inhibition of spindle formation) as summarized in TABLE 4, Figures 5A, 5B, 5C and 5D show representative fields of KSHV-infected DMVEC treated with PMOs as indicated, and visualized by CD31 staining.

Specifically, Figure 4A shows representative control (no PMO oligonucleotides) KSHV-infected DMVEC cultured as described herein above, and corresponds to 100% proliferation as presented in the growth inhibition assays summarized in TABLE 4.

Figure 4B illustrates representative RDC-1-specific PMO-treated KSHV-infected DMVEC, and corresponds to the 43% growth inhibition value (full phenotypic inhibition) as presented in TABLE 4.

Figure 4C illustrates representative KIAA0367-specific PMO-treated KSHV-infected DMVEC, and corresponds to the 28% growth inhibition value (intermediate phenotypic inhibition) as presented in TABLE 4.

Figure 4D illustrates representative MFAP-specific PMO-treated KSHV-infected DMVEC, and corresponds to the 11% growth inhibition value (no phenotypic inhibition) as presented in TABLE 4.

Therefore, according to the present invention, the extent of PMO-mediated inhibition of KSHV-induced proliferation (% growth inhibition) correlates with the corresponding phenotype inhibition values (full, intermediate and no inhibition).

KSHV-induced genes excluded as therapeutic targets by PMO antisense validation protocol. The above Examples show that with respect to particular identified KSHV-induced genes (*e.g.*, I-FACTOR, LMO2 and MFAP3), treatment of KSHV-infected DMVEC with the respective antisense PMO oligonucleotides had little or no affect on KSHV-induced spindle cell formation, despite the effectiveness of such antisense agents in mediating silencing of the respective gene sequences. This was not unexpected, because KSHV-related modulation of some cellular genes would reasonably be expected to be either ancillary to, or downstream from the regulatory cascades leading to spindle cell formation.

Significantly, the identification of KSHV-induced gene sequences which, upon silencing, have no effect on spindle formation provides internal (apart from the use of particular control PMO antisense molecules, etc.) confirmation that the inventive gene-silencing mediated preclusion of spindle cell formation is not mediated through ancillary or non-sequence-specific secondary effects of the respective PMO antisense molecules.

Therefore, data presented herein describes, teaches and supports the use of sequence-specific PMO antisense oligomers, *inter alia*, for **(i)** validation of therapeutic 'targets'; that is, for identification of KSHV-induced cellular gene products *required* for KSHV-induced cellular phenomena (*e.g.* spindle cell formation, transformation, angiogenesis, cancer, etc.), and **(ii)** as effective, non-toxic inhibitors of such validated therapeutic targets for modulation of KSHV infection and treatment of KSHV-induced proliferative disorders such as cancer. This utility is especially valuable where the particular gene products otherwise lack suitable art-recognized small molecule inhibitors.

Additionally, in view of deficiencies in the prior art teachings, these data emphasize the significance of functional validation of KSHV-induced gene sequences, according to the present invention to provide targets, compositions and methods having utility for blocking KSHV infection and for treating cancer.

EXAMPLE 4

(A Novel NUDE Mouse Model For Kaposi's Sarcoma Pathogenesis)

KSHV studies in vitro. Applicants have herein developed an *in vitro* system in which DMVEC are transformed to spindle cells that form 3-dimensional growth foci when infected with KSHV, and have used DNA microarray analysis to identify cellular genes whose

expression patterns are significantly altered by virus infection. Further, applicants have herein shown that silencing the virus-induced expression of certain cellular genes with antisense oligonucleotides leads to inhibition of spindle cell formation and foci development in the described *in vitro* cell culture model. According to the present invention, cellular genes inappropriately activated by KSHV infection contribute to cancer formation and are novel therapeutic targets for KS treatment.

Spindle cells cultured from KS tumors do not stably maintain the KSHV genome if KS tissue explants are cultured *ex vivo* (Aluigi et al., *Res Virol* 147(5):267-75, 1996; and Ambroziak et al., *Science* 268(5210):582-3, 1995). Thus, the development of endothelial cell-based *in vitro* models of KSHV infection that accurately reflect both the virus lifecycle and the disease phenotype is important for understanding KS tumorigenesis. Applicants were the first to successfully describe such a system based on infection of dermal microvascular endothelial cells (DMVEC) (Moses et al., *J. Virol.* 73(8):6892-6902, 1999). In this model, the majority of DMVEC become latently infected, cells develop a phenotype reminiscent of KS spindle cells, and lose contact inhibition when cultured post confluence (*see also* Ciufo, et al., *J Virol* 75(12):5614-26, 2001; and Lagunoff, et al., *J Virol* 76(5):2440-8, 2002).

In vivo studies. A limited number of murine models for KS have previously been described. KS cell lines isolated from AIDS/KS patients have been used to produce tumors of human origin in immunodeficient mice (Lunardi-Iskandar, et al., *J Natl. Cancer Inst.* 5:974-981, 1995; and Albini et al., *FASAEB J.* 13:647-655, 1999). These human KS cell lines have also been used to promote the growth of angioproliferative lesions of mouse origin by secretion of factors such as VEGF and bFGF (Ensoli, et al., *Nature* 371:674-676, 1994; and Samaniego, et al., *J. Immunol.* 158:1887-1897, 1997). However, these models are somewhat limited by the fact that while the utilized KS cell lines induce angiogenic lesions, these cells do not maintain the KSHV genome over the long-term.

Recently, KS-like tumors have been generated in mice transgenic for the avian leucosis virus (ALV) receptor, TVA; the mice were infected with ALV vectors expressing KSHV genes (Montaner, et al., *Cancer Cell.* 3:23-36, 2003). However, this model is limited by the fact that the induced tumors are of mouse origin and were induced via retroviral vectors encoding KSHV oncogenes.

Therefore, there is a need in the art to create tumors of human origin that maintain the entire KSHV genome, and thus more accurately reflect the cellular and viral interactions occurring in KS lesions. There is a need in the art for an *in vivo* model that can be used to

directly examine the role of virus-induced cellular proteins in driving tumor establishment and/or growth. There is a need in the art for an *in vivo* model system to screen and test novel KS drugs. There is a need in the art for an *in vivo* model system wherein the cells contain the KSHV genome, so that inhibitors of virus replication as well as gene expression can be screened/tested.

Irradiation model; mice were irradiated to impair immune function. In particular embodiments of the present invention, BALB/c mice were subjected to irradiation to temporarily decrease immune function and ablate the tumor rejection response. Mock- and KSHV-infected DMVEC (3×10^6 cells/injection) were suspended in serum-free culture medium, mixed with 0.2 ml (1:1) of matrigel and injected subcutaneously into the tail base. 10 days later, mice were humanely euthanized according to an OHSU IACUC-approved protocol and matrigel plugs were excised. One half of each plug was placed into tissue culture for phase microscopy observation after which it was used for extraction of cellular DNA and PCR for the KSHV Bam330 fragment to verify maintenance of the KSHV genome. The other half was embedded in paraffin, sectioned and stained with a rabbit anti-human polyclonal antibody against heme-oxygenase 1, a cellular protein induced by KSHV infection of DMVEC and implicated in the angiogenic process (McAllister, et al., *Blood* In press, 2004).

Results. Matrigel plugs excised from the control mouse injected with mock-infected DMVEC contained only degenerating cell clumps. In obvious contrast, KSHV-infected cells had developed into a distinct vascular network running through the 3-dimensional matrigel matrix. 233bp of KSHV ORF26 (Bam300 fragment) was amplified exclusively from DNA extracted from within the KSHV-infected DMVEC matrigel plug, indicating maintenance of the KSHV genome. Finally, immunohistochemical staining of paraffin-embedded matrigel sections revealed reactivity to human HO-1 in vascular threads within the KSHV-infected matrigel sections.

Therefore, according to the present invention, KSHV-infected DMVEC showed a preferential tendency to survive and undergo angiogenesic growth in immunodeficient (irradiated) mice.

Novel Nude mouse model. According to the present invention, applicants' KSHV-infected DMVEC model has further utility to induce KS-like tumors in immunodeficient mice.

According to the present invention, a nude mouse model for KS is developed by implanting KSHV-transformed DMVEC into immunodeficient (nude) mice.

According to the present invention, DMVEC are treated prior to implantation into nude mice to inhibit the expression of virus-induced genes, whereby the tumorigenic potential of the treated implants is evaluated.

According to the present invention, the use of nude mice, allows for more robust tumor growth, and allows for the efficient growth of KSHV-infected human cells in the mouse model, development of KS like tumors, and further validation of anti KS therapies.

Specifically, according to particular embodiments of the present invention, Nude mice (*Foxn1*^{nu}) on a BALB/cByJ genetic background are obtained from The Jackson Laboratory (Bar Harbor, Maine). Because the forkhead box N1 gene mutation disrupts thymic function, nude mice exhibit T cell deficiency with some defects in B cell development. The activity of macrophages, antigen presenting cells and NK cells is unaffected, and reduces susceptibility to murine pathogens. Nude mice have been widely used for the growth of human tumors, and the lack of hair allows visualization of sub-cutaneous tumors.

According to the present invention, mice receive subcutaneous injections at the tail base, where the injection material consists of KSHV infected human dermal microvascular endothelial cells (DMVEC) (3×10^6 cells/injection) that are suspended in serum-free culture medium and mixed with 0.2 ml (1:1) of matrigel. DMVEC are infected with KSHV at least two weeks prior to inoculation, to allow establishment of latent infection in the majority of cells (Moses et al., *J. Virol.* 73(8):6892-6902, 1999; and Moses, et al., *J. Virol.* 76(16):8383-8399, 2002). Negative controls include animals injected with uninfected DMVEC in matrigel or with matrigel alone. As a positive control, the fibrosarcoma HT1080 (ATCC # CRL-12012) that readily forms tumors in nude mice is used.

In some experiments, DMVEC are loaded with antisense oligonucleotides (PMOs) to inhibit expression of specific cellular genes 24 hours prior to inoculation (Moses, et al., *Ann NY Acad Sci* 975:1-12, 2002). Briefly, cells are incubated with a PMO-loading reagent complex for three hours, rinsed and cultured overnight prior to resuspension in matrigel and inoculation. Parallel cultures are maintained *in vitro* to verify PMO uptake and efficiency of gene silencing. Alternatively, siRNA agents and methods are used to inhibit expression of specific cellular sequences.

According to the present invention, mice are observed and weighed daily. Caliper measurements of tumor size are recorded daily. At days 7 and 14 post-inoculation, mice are euthanized. Lesions at the site of inoculation are macroscopically examined, excised, measured and weighed. If no lesions are present, equivalent tissue areas around the injection site a re excised. Excised tissue is divided into thirds and is treated as follows: (i) fixed in formalin for

histologic examination following H&E staining; (ii) frozen in OCT for immunohistochemistry; (iii) processed for RNA extraction and pPCR analysis. Protein and mRNA evaluations include cellular and viral targets.

Additional organs such as spleen and draining lymph node are processed and analyzed. Mice are examined for metastases to the gut, liver and kidney and such tissues are harvested if warranted.

All animals are euthanized at the pre-assigned times. Animals are euthanized immediately if they exhibit any signs of undue tumor burden including: a tumor that exceeding 2 cm or 10% of body weight; ulceration of tumor, tumor impeding ambulation or ability to obtain food or water; if the animal exhibits signs or pain or distress; or if the animal is cachexic or moribund. A protocol for these studies is approved by the OSHU IACUC Protocol # A924.

According to the present invention, mice inoculated with HT1080 fibrosarcoma cells form tumors and serve as a positive control. According to the present invention, mice inoculated with KSHV-infected DMVEC develop tumors at the injection site within 5-7 days, whereas no tumors develop in mice inoculated with uninfected DMVEC or with matrigel alone.

According to the present invention, mice inoculated with KSHV-DMVEC in which expression of KSHV genes has been inhibited by PMO treatment (or siRNA treatment) show different degrees of tumor inhibition, depending on the relative importance of the cellular gene that is targeted. A central role for c-Kit in KS transformation has been demonstrated *in vitro*, and, according to the present invention, tumor formation is inhibited *in vivo* when c-Kit expression is inhibited. According to the present invention, the performance of other PMOs in this *in vivo* system likewise confirms the role of the targeted cellular gene in KS tumorigenesis, and further validates the therapeutic approach.

According to the present invention, mice are inoculated with KSHV-DMVEC in which PMO treatment (or siRNA treatment) is used to inhibit expression of at least one KSHV-induced cellular gene sequence selected from the group disclosed herein consisting of: RDC-1 (GPCR RDC1); IGFBP2 (insulin-like growth factor binding protein 2); FLJ14103 (hypothetical protein FLJ14103); Neuritin; KIT (c-KIT); LOX (lysyl oxidase preprotein); Nov (nov precursor); KIAA0367 (KIAA0367 protein); INSR (Insulin receptor); and ANGPTL2 (angiopoietin-like 2 precursor), wherein inhibition of tumors, relative to controls, is shown, and whereby the targeted sequences are further validated and whereby therapeutic utility is further confirmed.

CLAIMS

1. A method for identification of agents or compounds useful to modulate KSHV infection, comprising:

(a) contacting one or more KSHV-infected cells with a test agent or compound;

(b) measuring in the one or more cells, and using a suitable assay, expression of a *validated* KSHV-induced cellular gene or gene product, wherein a *validated* gene or gene product is a gene or gene product the expression of which is required, at least to some extent, for KSHV infection or KSHV-mediated effects on cellular proliferation and phenotype; and

(c) determining, relative to one or more control cells not contacted with the test agent or compound, whether the test agent or compound inhibits the *validated* gene or gene product expression, whereby agents or compounds that inhibit the *validated* gene or gene product expression are identified as agents or compounds useful to modulate KSHV infection.

2. The method of claim 1, wherein measuring expression of a *validated* KSHV-induced cellular gene or gene product is by measuring the presence or amount at least one of the corresponding mRNA or the protein product encoded thereby.

3. The methods of any one of claims 1 or 2, further comprising testing of the agents or compounds that inhibit the *validated* KSHV-induced cellular gene or gene product expression for the ability to modulate at least one of KSHV infection, or KSHV-mediated effects on cellular proliferation or phenotype.

4. The methods of any one of claims 1, 2 or 3, wherein the KSHV-infected cells are KSHV-infected dermal microvascular endothelial cells (DMVEC).

5. The method of any one of claims 1-4, comprising measuring the expression of a plurality of *validated* KSHV-induced cellular genes or gene products.

6. The method of any one of claims 1-5, wherein at least one of measuring or determining comprises use of high-throughput microarray methods.

7. The method or assay of any one of claims 1 through 6, wherein the validated KSHV-induced cellular genes or gene products correspond to one or more nucleic acid sequences selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29, for the RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT (c-kit), LOX, NOV and ANGPTL2 cDNA sequences, respectively.

8. The methods of any one of claims 1 through 6, wherein the validated KSHV-induced cellular genes or gene products correspond to one or more amino acid sequences selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 26, 28 and 30, for the

RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT (c-kit), LOX, NOV and ANGPTL2 protein sequences, respectively.

9. A diagnostic or prognostic assay for KSHV infection, comprising:

(a) obtaining a cell sample from a subject having, or suspected of having KSHV;

(b) measuring in the sample, and using a suitable assay, expression of a *validated* KSHV-induced cellular gene or gene product, wherein a *validated* gene or gene product is a gene or gene product the expression of which is required, at least to some extent, for KSHV infection; and

(c) determining, based on the measuring, and relative to that of non-KSHV-infected control cells, whether expression of the *validated* KSHV-induced cellular gene or gene product is induced, whereby a diagnosis or prognosis is, at least in part, afforded.

10. The assay of claim 9, comprising measuring the expression of a plurality of *validated* KSHV-induced cellular genes or gene products.

11. The assay of any one of claims 9 or 10, wherein at least one of measuring or determining comprises use of high-throughput microarray methods.

12. The assay of any one of claims 9, 10 or 11, wherein the validated KSHV-induced cellular genes or gene products correspond to one or more nucleic acid sequences selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29, for the RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT (c-kit), LOX, NOV and ANGPTL2 cDNA sequences, respectively.

13. The assay of any one of claims 9, 10 or 11, wherein the validated KSHV-induced cellular genes or gene products correspond to one or more amino acid sequences selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 26, 28 and 30, for the RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT (c-kit), LOX, NOV and ANGPTL2 protein sequences, respectively.

14. A method of inhibiting at least one of: KSHV-induced cellular gene expression or encoded biological activity; KSHV infection; or KSHV-mediated effects on cellular proliferation and phenotype, comprising introducing into, or expressing within a KSHV-infected human cell at least one of an antisense, siRNA or ribozyme agent specific for a *validated* KSHV-induced cellular gene sequence, and in an amount sufficient to inhibit, at least to some extent, expression of the *validated* KSHV-induced cellular gene sequence, wherein a *validated* KSHV-induced cellular gene sequence is a nucleic acid sequence the expression of which is required, at least to some extent, for the KSHV-induced cellular gene expression or encoded biological activity, the KSHV infection, or the KSHV-mediated effects on cellular proliferation and phenotype.

15. The method of claim 14, wherein inhibiting the KSHV-mediated effects on cellular proliferation and phenotype comprises inhibiting proliferation or development of cancer cells.

16. The method of any one of claims 14 or 15, wherein the *validated* KSHV-induced cellular gene sequence is that corresponding to a nucleic acid sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29, for the RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT (c-kit), LOX, NOV and ANGPTL2 cDNA sequences, respectively.

17. The method of any one of claims 14-16, wherein the antisense agent specific for a *validated* KSHV-induced cellular gene sequence comprises a nucleic acid sequence of at least 18 contiguous bases in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27, 29, and sequences complementary thereto.

18. The method of any one of claims 14-17, wherein the antisense agent specific for a *validated* KSHV-induced cellular gene sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS:15-24, 31-32 and 33.

19. The method of any one of claims 14-18, wherein the *validated* KSHV-induced cellular gene sequence-specific antisense agent comprises a Phosphorodiamidate Morpholino Oligomers (PMO) antisense oligonucleotide specific for the *validated* KSHV-induced cellular gene sequence.

20. A method for inhibiting or treating KSHV-infection in a subject, or for treating KSHV-related neoplastic disease, comprising administering to the subject a therapeutically effective amount of at least one of an antisense, siRNA or ribozyme agent specific for a *validated* KSHV-induced cellular gene sequence, wherein the *validated* KSHV-induced cellular gene sequence is a nucleic acid sequence the expression of which is required, at least to some extent, for the KSHV-infection or the KSHV-related neoplastic disease.

21. The method of claim 20, wherein the *validated* KSHV-induced cellular gene sequence is that corresponding to a nucleic acid sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29, for the RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT (c-kit), LOX, NOV and ANGPTL2 cDNA sequences, respectively.

22. The method of any one of claims 20 or 21, wherein the antisense agent specific for a *validated* KSHV-induced cellular gene sequence comprises a nucleic acid sequence of at least 18 contiguous bases in length that is complementary to, or hybridizes under moderately

stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27, 29, and sequences complementary thereto.

23. The method of any one of claims 20-22, wherein the antisense agent specific for a *validated* KSHV-induced cellular gene sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS:15-24, 31-32 and 33.

24. The method of any one of claims 20-23, wherein the *validated* KSHV-induced cellular gene sequence-specific antisense agent comprises a Phosphorodiamidate Morpholino Oligomers (PMO) antisense oligonucleotide specific for the *validated* KSHV-induced cellular gene sequence.

25. Use of an inhibitor of *validated* KSHV-induced gene or gene product expression to prepare a medicament for modulating at least one of KSHV infection, KSHV-mediated effects on cellular proliferation or phenotype, or KSHV-related neoplastic disease, and wherein the inhibitor comprises at least one of an antisense, siRNA or ribozyme agent specific for the *validated* KSHV-induced gene or gene product.

26. The use of claim 25, wherein the validated KSHV-induced cellular genes or gene products correspond to one or more nucleic acid sequences selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27 and 29, for the RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT (c-kit), LOX, NOV and ANGPTL2 cDNA sequences, respectively.

27. The use of claim 25, wherein the validated KSHV-induced cellular genes or gene products correspond to one or more amino acid sequences selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 14, 26, 28 and 30, for the RDC-1, IGFBP2, FLJ14103, KIAA0367, Neuritin, INSR, KIT (c-kit), LOX, NOV and ANGPTL2 protein sequences, respectively.

28. The use of any one of claims 25, 26 or 27, wherein the inhibitor of *validated* KSHV-induced gene or gene product expression comprises an antisense agent specific to the *validated* KSHV-induced gene or gene product.

29. The use of any one of claims 25-28, wherein the antisense agent specific for a *validated* KSHV-induced cellular gene sequence comprises a nucleic acid sequence of at least 18 contiguous bases in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27, 29, and sequences complementary thereto.

30. The use of any one of claims 25-29, wherein the antisense agent specific for a *validated* KSHV-induced cellular gene sequence comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS:15-24, 31-32 and 33.

31. The use of any one of claims 25-30, wherein the *validated* KSHV-induced cellular gene sequence-specific antisense agent comprises a Phosphorodiamidate Morpholino Oligomers (PMO) antisense oligonucleotide specific for the *validated* KSHV-induced cellular gene sequence.

32. An antisense oligonucleotide, siRNA agent, or a ribozyme agent comprising a sequence of about 10 to about 35 contiguous nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27, 29, and sequences complementary thereto, wherein the antisense oligonucleotide, siRNA agent, or a ribozyme agent is effective to inhibit cellular expression, at least to some degree, of the respective KSHV-induced human cellular gene product.

33. A recombinant expression vector, comprising a transcriptional initiation region and a sequence encoding a KSHV-induced gene-specific antisense oligonucleotide, siRNA agent, or ribozyme agent a sequence of about 10 to about 35 contiguous nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 25, 27, 29, and sequences complementary thereto.

34. An *in vivo* mouse model for KSHV infection and KSHV-related conditions, comprising introduction of KSHV-infected human dermal microvascular endothelial cells (DMVEC) into a immunodeficient NUDE mouse strain.

34. The mouse model of claim 34, wherein the NUDE mouse strain is $Foxn1^{nu}$ on a BALB/cByJ genetic background.

35. The mouse model of any one of claims 34 or 35, wherein KS-like tumors are induced by introduction of KSHV-infected human dermal microvascular endothelial cells (DMVEC).

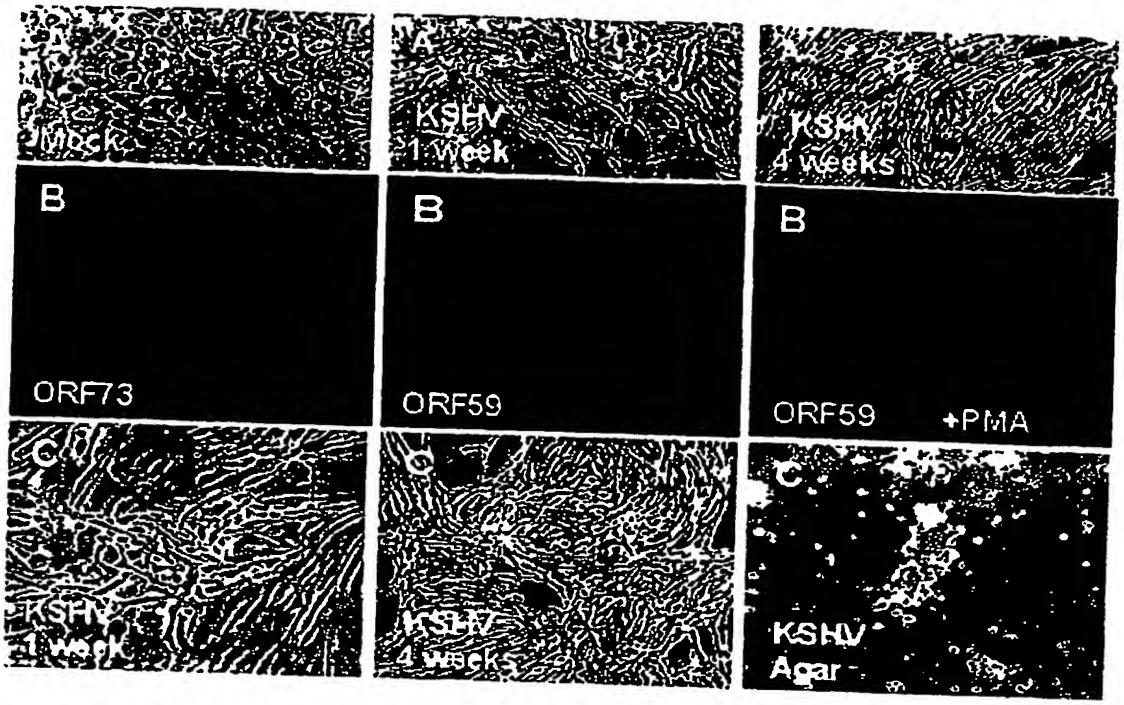

A
Mock
A
KSHV
1 week
A
KSHV
4 weeks
B
ORF73
B
ORF59
B
ORF59 +PMA
C
KSHV
1 week
C
KSHV
4 week
C
KSHV
Agar

FIG. 1A, B, C

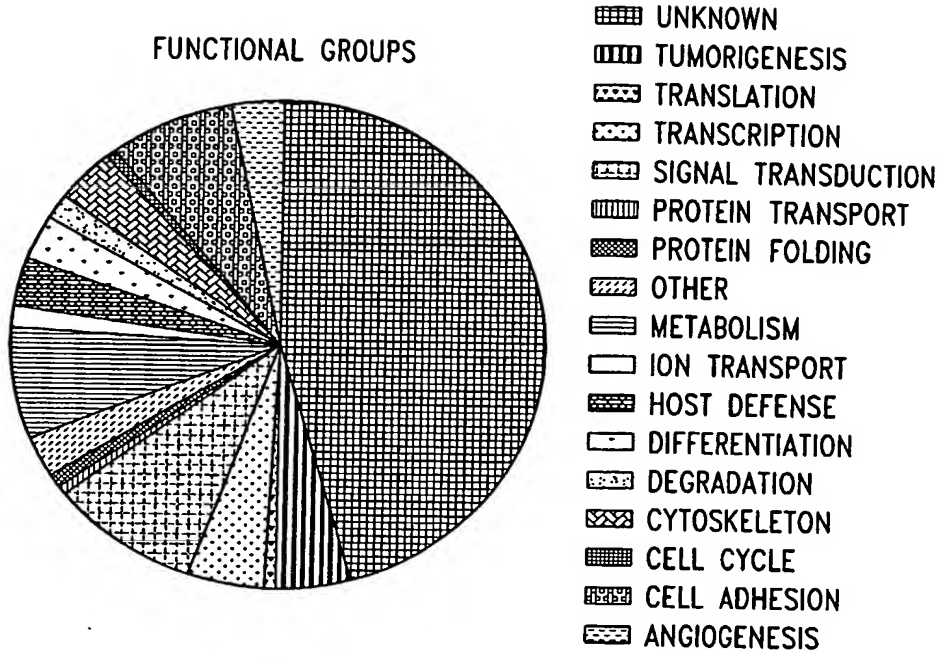

FUNCTIONAL GROUPS
UNKNOWN
TUMORIGENESIS
TRANSLATION
TRANSCRIPTION
SIGNAL TRANSDUCTION
PROTEIN TRANSPORT
PROTEIN FOLDING
OTHER
METABOLISM
ION TRANSPORT
HOST DEFENSE
DIFFERENTIATION
DEGRADATION
CYTOSKELETON
CELL CYCLE
CELL ADHESION
ANGIOGENESIS

Fig. 2

PMO inhibition of KSHV phenotype

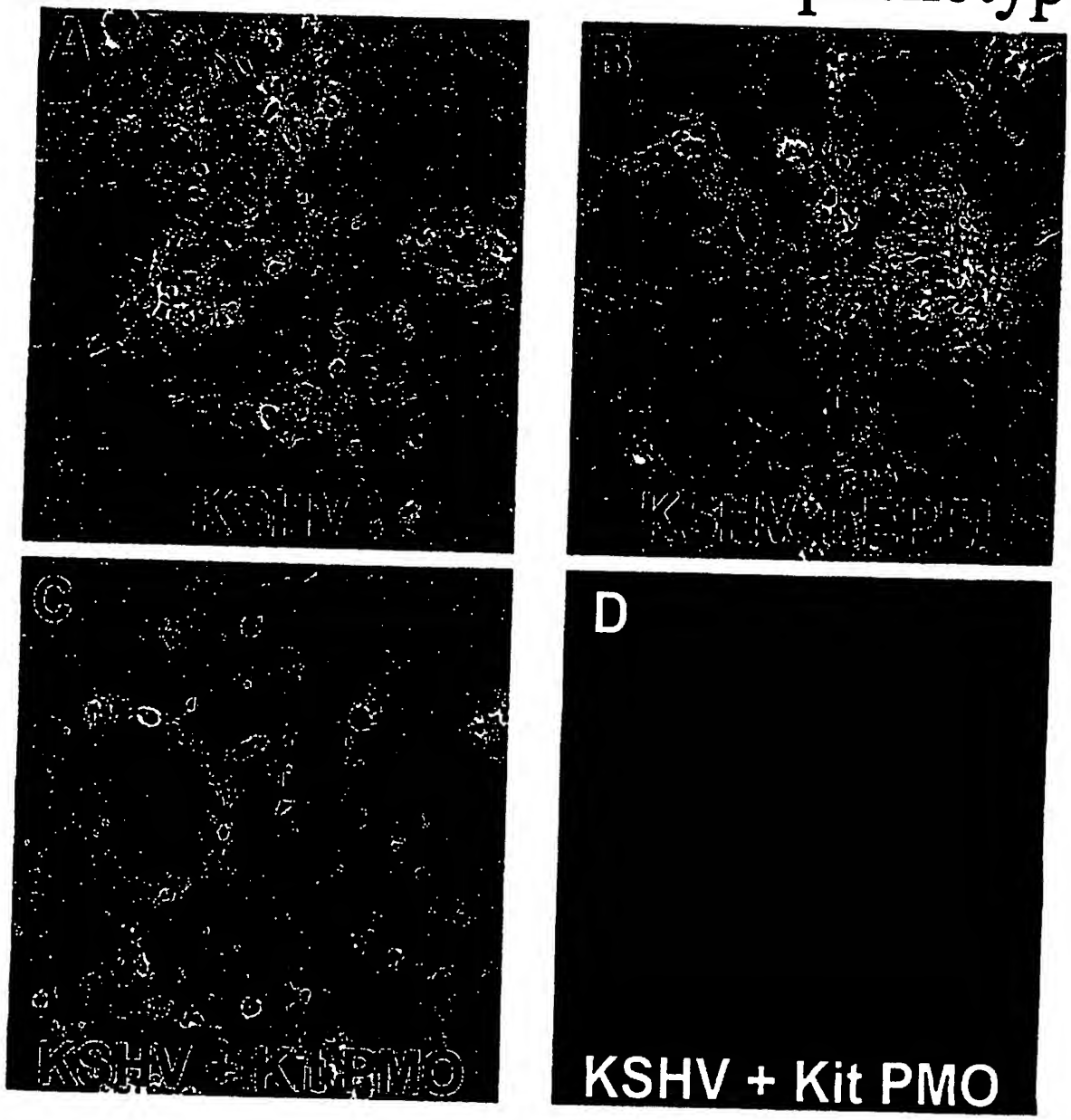
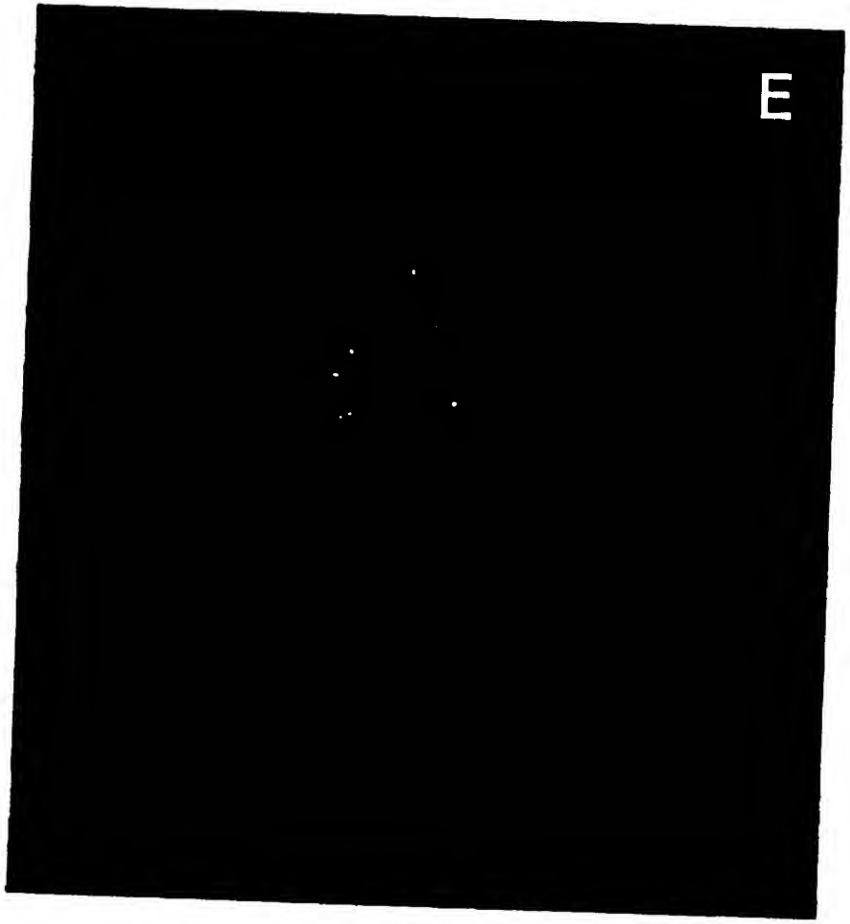


FIG. 3A

PMO Inhibition of c-kit Expression



c-Kit protein

FITC-tagged PMO

FIG. 3B

Control (No PMO)

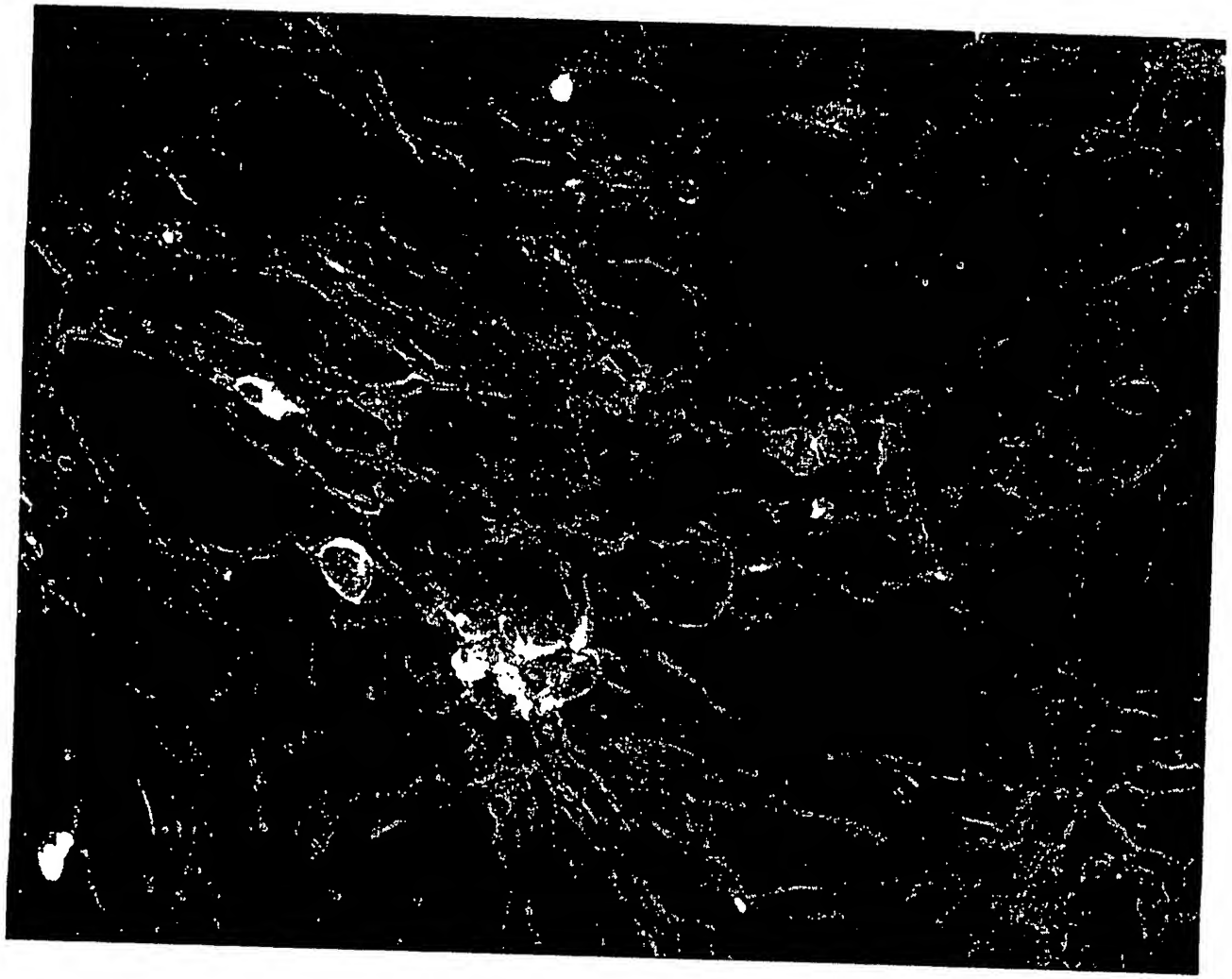


FIG. 4A

Full Inhibition (RDC-1)

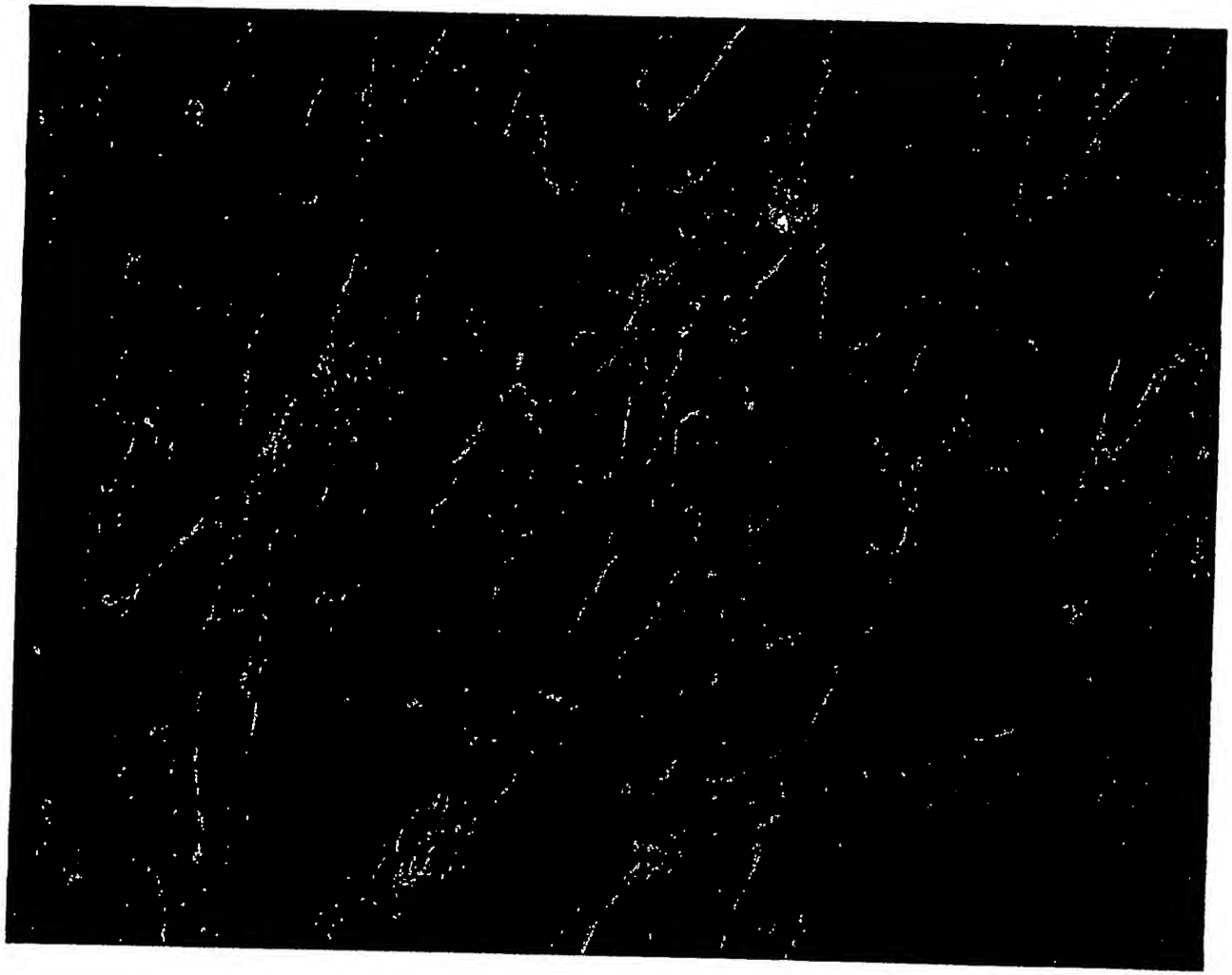


FIG. 4B

Intermediate inhibition (KIAA)

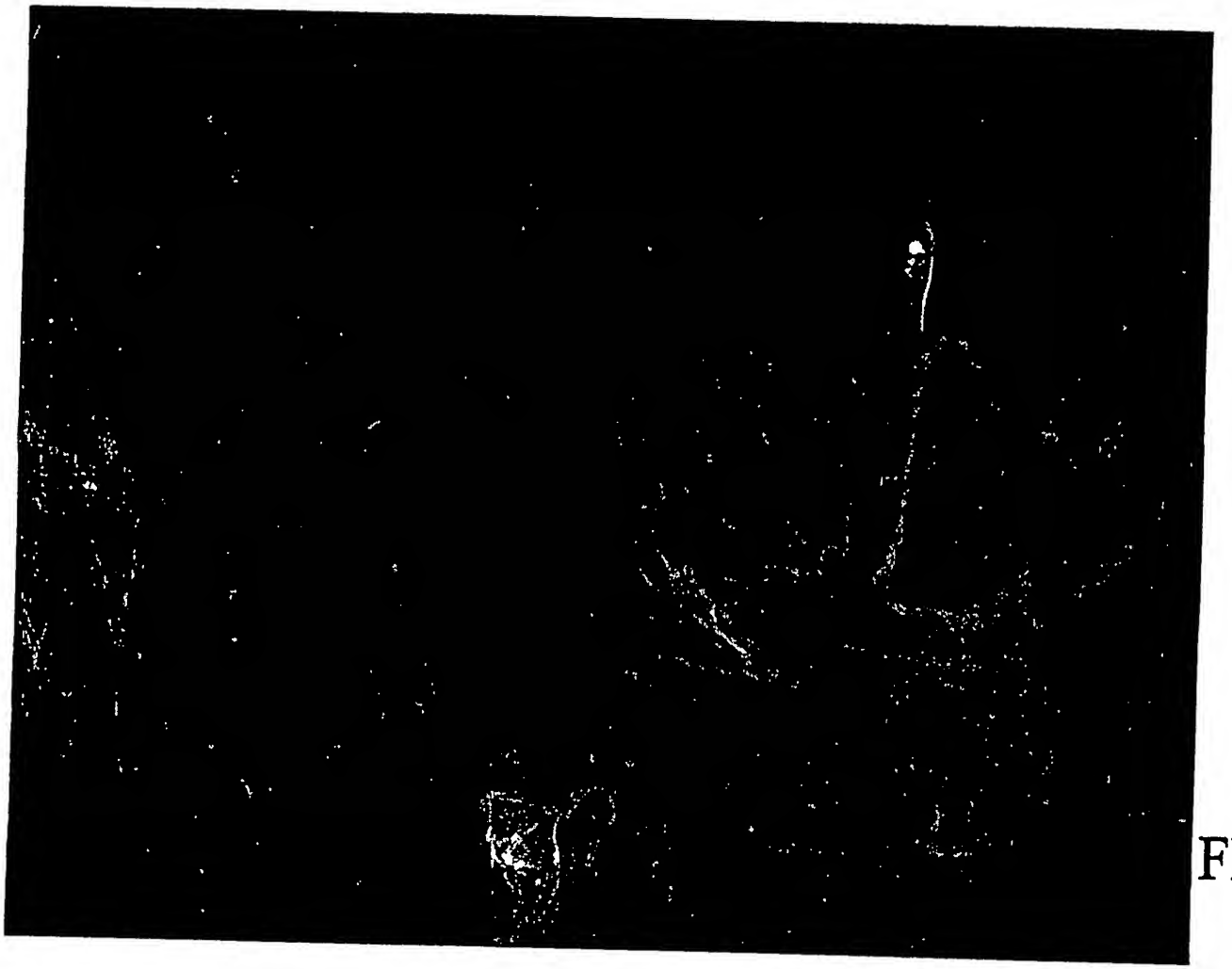


FIG. 4C

No Inhibition (MFAP)

FIG. 4D

49321-111.ST25.txt
SEQUENCE LISTING

<110> Oregon Health & Science University
Moses, Ashlee
Fruh, Klaus
King, Jeffrey S.
Hicks, James B.
Raggo, Camilo
Nelson, Jay

<120> METHODS OF TREATMENT AND DIAGNOSIS OF KAPOSI'S SARCOMA (KS) AND KS RELATED DISEASES

<130> 49321-111

<150> US 60/438,343
<151> 2003-01-06

<150> US 60/473,246
<151> 2003-05-22

<160> 33

<170> PatentIn version 3.2

<210> 1
<211> 2035
<212> DNA
<213> homo sapiens

<220>
<221> CDS
<222> (152)..(1240)

<400> 1
tgcaagtctg cagccagcag agctcacagt tgttgcaaag tgctcagcac taagggagcc 60

agcgcacagc acagccagga aggcgagcga gcccagccag cccagccagc ccagccagcc 120

cggaggtcat ttgattgccc gcctcagaac g atg gat ctg cat ctc ttc gac 172
Met Asp Leu His Leu Phe Asp
1 5

tac tca gag cca ggg aac ttc tcg gac atc agc tgg cca tgc aac agc 220
Tyr Ser Glu Pro Gly Asn Phe Ser Asp Ile Ser Trp Pro Cys Asn Ser
10 15 20

agc gac tgc atc gtg gtg gac acg gtg atg tgt ccc aac atg ccc aac 268
Ser Asp Cys Ile Val Val Asp Thr Val Met Cys Pro Asn Met Pro Asn
25 30 35

aaa agc gtc ctg ctc tac acg ctc tcc ttc att tac att ttc atc ttc 316
Lys Ser Val Leu Leu Tyr Thr Leu Ser Phe Ile Tyr Ile Phe Ile Phe
40 45 50 55

gtc atc ggc atg att gcc aac tcc gtg gtg gtc tgg gtg aat atc cag 364
Val Ile Gly Met Ile Ala Asn Ser Val Val Val Trp Val Asn Ile Gln
60 65 70

gcc aag acc aca ggc tat gac acg cac tgc tac atc ttg aac ctg gcc 412
Ala Lys Thr Thr Gly Tyr Asp Thr His Cys Tyr Ile Leu Asn Leu Ala
75 80 85

```
att gcc gac ctg tgg gtt gtc ctc acc atc cca gtc tgg gtg gtc agt      460
Ile Ala Asp Leu Trp Val Val Leu Thr Ile Pro Val Trp Val Val Ser
        90                  95                  100

ctc gtg cag cac aac cag tgg ccc atg ggc gag ctc acg tgc aaa gtc      508
Leu Val Gln His Asn Gln Trp Pro Met Gly Glu Leu Thr Cys Lys Val
    105                 110                 115

aca cac ctc atc ttc tcc atc aac ctc ttc ggc agc att ttc ttc ctc      556
Thr His Leu Ile Phe Ser Ile Asn Leu Phe Gly Ser Ile Phe Phe Leu
120                 125                 130                 135

acg tgc atg agc gtg gac cgc tac ctc tcc atc acc tac ttc acc aac      604
Thr Cys Met Ser Val Asp Arg Tyr Leu Ser Ile Thr Tyr Phe Thr Asn
                140                 145                 150

acc ccc agc agc agg aag aag atg gta cgc cgt gtc gtc tgc atc ctg      652
Thr Pro Ser Ser Arg Lys Lys Met Val Arg Arg Val Val Cys Ile Leu
            155                 160                 165

gtg tgg ctg ctg gcc ttc tgc gtg tct ctg cct gac acc tac tac ctg      700
Val Trp Leu Leu Ala Phe Cys Val Ser Leu Pro Asp Thr Tyr Tyr Leu
        170                 175                 180

aag acc gtc acg tct gcg tcc aac aat gag acc tac tgc cgg tcc ttc      748
Lys Thr Val Thr Ser Ala Ser Asn Asn Glu Thr Tyr Cys Arg Ser Phe
    185                 190                 195

tac ccc gag cac agc atc aag gag tgg ctg atc ggc atg gag ctg gtc      796
Tyr Pro Glu His Ser Ile Lys Glu Trp Leu Ile Gly Met Glu Leu Val
200                 205                 210                 215

tcc gtt gtc ttg ggc ttt gcc gtt ccc ttc tcc att atc gct gtc ttc      844
Ser Val Val Leu Gly Phe Ala Val Pro Phe Ser Ile Ile Ala Val Phe
                220                 225                 230

tac ttc ctg ctg gcc aga gcc atc tcg gcg tcc agt gac cag gag aag      892
Tyr Phe Leu Leu Ala Arg Ala Ile Ser Ala Ser Ser Asp Gln Glu Lys
            235                 240                 245

cac agc agc cgg aag atc atc ttc tcc tac gtg gtg gtc ttc ctt gtc      940
His Ser Ser Arg Lys Ile Ile Phe Ser Tyr Val Val Val Phe Leu Val
        250                 255                 260

tgc tgg ctg ccc tac cac gtg gcg gtg ctg ctg gac atc ttc tcc atc      988
Cys Trp Leu Pro Tyr His Val Ala Val Leu Leu Asp Ile Phe Ser Ile
    265                 270                 275

ctg cac tac atc cct ttc acc tgc cgg ctg gag cac gcc ctc ttc acg     1036
Leu His Tyr Ile Pro Phe Thr Cys Arg Leu Glu His Ala Leu Phe Thr
280                 285                 290                 295

gcc ctg cat gtc aca cag tgc ctg tcg ctg gtg cac tgc tgc gtc aac     1084
Ala Leu His Val Thr Gln Cys Leu Ser Leu Val His Cys Cys Val Asn
                300                 305                 310

cct gtc ctc tac agc ttc atc aat cgc aac tac agg tac gag ctg atg     1132
Pro Val Leu Tyr Ser Phe Ile Asn Arg Asn Tyr Arg Tyr Glu Leu Met
            315                 320                 325

aag gcc ttc atc ttc aag tac tcg gcc aaa aca ggg ctc acc aag ctc     1180
Lys Ala Phe Ile Phe Lys Tyr Ser Ala Lys Thr Gly Leu Thr Lys Leu
        330                 335                 340
```

330 335 340

atc gat gcc tcc aga gtc tca gag acg gag tac tct gcc ttg gag cag 1228
Ile Asp Ala Ser Arg Val Ser Glu Thr Glu Tyr Ser Ala Leu Glu Gln
345 350 355

agc acc aaa tga tctgccctgg agaggctctg ggacgggttt acttgttttt 1280
Ser Thr Lys
360

gaacagggtg atgggcccta tggttttcta gagcaaagca aagtagcttc gggtcttgat 1340

gcttgagtag agtgaagagg ggagcacgtg ccccctgcat ccattctctc tttctcttga 1400

tgacgcagct gtcatttggc tgtgcgtgct gacagttttg caacaggcag agctgtgtcg 1460

cacagcagtg ctgtgcgtca gagccagctg aggacaggct tgcctggact tctgtaagat 1520

aggattttct gtgtttcctg aatttttat atggtgattt gtatttaaat tttaagactt 1580

tattttctca ctattggtgt accttataaa tgtatttgaa agttaaatat attttaaata 1640

ttgtttggga ggcatagtgc tgacatatat tcagagtgtt gtagttttaa ggttagcgtg 1700

acttcagttt tgactaagga tgacactaat tgttagctgt tttgaaatta tatatatata 1760

aatatatata aatatataaa tatatgccag tcttggctga aatgttttat ttaccatagt 1820

tttatatctg tgtggtgttt tgtaccggca cgggatatgg aacgaaaact gctttgtaat 1880

gcagtttgtg acattaatag tattgtaaag ttacatttta aaataaacaa aaaactgttc 1940

tggactgcaa atctgcacac acaacgaaca gttgcatttc agagagttct ctcaatttgt 2000

aagttatttt tttttaataa agatttttgt ttcct 2035

<210> 2
<211> 362
<212> PRT
<213> homo sapiens

<400> 2

Met Asp Leu His Leu Phe Asp Tyr Ser Glu Pro Gly Asn Phe Ser Asp
1 5 10 15

Ile Ser Trp Pro Cys Asn Ser Ser Asp Cys Ile Val Val Asp Thr Val
20 25 30

Met Cys Pro Asn Met Pro Asn Lys Ser Val Leu Leu Tyr Thr Leu Ser
35 40 45

Phe Ile Tyr Ile Phe Ile Phe Val Ile Gly Met Ile Ala Asn Ser Val
50 55 60

Val Val Trp Val Asn Ile Gln Ala Lys Thr Thr Gly Tyr Asp Thr His
65 70 75 80

Cys Tyr Ile Leu Asn Leu Ala Ile Ala Asp Leu Trp Val Val Leu Thr
85 90 95

Ile Pro Val Trp Val Val Ser Leu Val Gln His Asn Gln Trp Pro Met
100 105 110

Gly Glu Leu Thr Cys Lys Val Thr His Leu Ile Phe Ser Ile Asn Leu
115 120 125

Phe Gly Ser Ile Phe Phe Leu Thr Cys Met Ser Val Asp Arg Tyr Leu
130 135 140

Ser Ile Thr Tyr Phe Thr Asn Thr Pro Ser Ser Arg Lys Lys Met Val
145 150 155 160

Arg Arg Val Val Cys Ile Leu Val Trp Leu Leu Ala Phe Cys Val Ser
165 170 175

Leu Pro Asp Thr Tyr Tyr Leu Lys Thr Val Thr Ser Ala Ser Asn Asn
180 185 190

Glu Thr Tyr Cys Arg Ser Phe Tyr Pro Glu His Ser Ile Lys Glu Trp
195 200 205

Leu Ile Gly Met Glu Leu Val Ser Val Val Leu Gly Phe Ala Val Pro
210 215 220

Phe Ser Ile Ile Ala Val Phe Tyr Phe Leu Leu Ala Arg Ala Ile Ser
225 230 235 240

Ala Ser Ser Asp Gln Glu Lys His Ser Ser Arg Lys Ile Ile Phe Ser
245 250 255

Tyr Val Val Val Phe Leu Val Cys Trp Leu Pro Tyr His Val Ala Val
260 265 270

Leu Leu Asp Ile Phe Ser Ile Leu His Tyr Ile Pro Phe Thr Cys Arg
275 280 285

Leu Glu His Ala Leu Phe Thr Ala Leu His Val Thr Gln Cys Leu Ser
290 295 300

Leu Val His Cys Cys Val Asn Pro Val Leu Tyr Ser Phe Ile Asn Arg
305 310 315 320

Asn Tyr Arg Tyr Glu Leu Met Lys Ala Phe Ile Phe Lys Tyr Ser Ala
325 330 335

Lys Thr Gly Leu Thr Lys Leu Ile Asp Ala Ser Arg Val Ser Glu Thr
340 345 350

Glu Tyr Ser Ala Leu Glu Gln Ser Thr Lys
355 360

<210> 3
<211> 1421
<212> DNA
<213> homo sapiens

<220>
<221> CDS
<222> (115)..(1092)

<400> 3
ggcgagggag gaggaagaag cggaggaggc ggctcccgcg ctcgcagggc cgtgccacct 60

gcccgcccgc ccgctcgctc gctcgcccgc cgcgccgcgc tgccgaccgc cagc atg 117
Met
1

ctg ccg aga gtg ggc tgc ccc gcg ctg ccg ctg ccg ccg ccg ccg ctg 165
Leu Pro Arg Val Gly Cys Pro Ala Leu Pro Leu Pro Pro Pro Pro Leu
5 10 15

ctg ccg ctg ctg ctg ctg cta ctg ggc gcg agt ggc ggc ggc ggc ggg 213
Leu Pro Leu Leu Leu Leu Leu Leu Gly Ala Ser Gly Gly Gly Gly Gly
20 25 30

gcg cgc gcg gag gtg ctg ttc cgc tgc ccg ccc tgc aca ccc gag cgc 261
Ala Arg Ala Glu Val Leu Phe Arg Cys Pro Pro Cys Thr Pro Glu Arg
35 40 45

ctg gcc gcc tgc ggg ccc ccg ccg gtt gcg ccg ccc gcc gcg gtg gcc 309
Leu Ala Ala Cys Gly Pro Pro Pro Val Ala Pro Pro Ala Ala Val Ala
50 55 60 65

gca gtg gcc gga ggc gcc cgc atg cca tgc gcg gag ctc gtc cgg gag 357
Ala Val Ala Gly Gly Ala Arg Met Pro Cys Ala Glu Leu Val Arg Glu
70 75 80

ccg ggc tgc ggc tgc tgc tcg gtg tgc gcc cgg ctg gag ggc gag gcg 405
Pro Gly Cys Gly Cys Cys Ser Val Cys Ala Arg Leu Glu Gly Glu Ala
85 90 95

tgc ggc gtc tac acc ccg cgc tgc ggc cag ggg ctg cgc tgc tat ccc 453
Cys Gly Val Tyr Thr Pro Arg Cys Gly Gln Gly Leu Arg Cys Tyr Pro
100 105 110

cac ccg ggc tcc gag ctg ccc ctg cag gcg ctg gtc atg ggc gag ggc 501
His Pro Gly Ser Glu Leu Pro Leu Gln Ala Leu Val Met Gly Glu Gly
115 120 125

act tgt gag aag cgc cgg gac gcc gag tat ggc gcc agc ccg gag cag 549
Thr Cys Glu Lys Arg Arg Asp Ala Glu Tyr Gly Ala Ser Pro Glu Gln
130 135 140 145

gtt gca gac aat ggc gat gac cac tca gaa gga ggc ctg gtg gag aac 597

```
Val Ala Asp Asn Gly Asp Asp His Ser Glu Gly Gly Leu Val Glu Asn
                150                 155                 160

cac gtg gac agc acc atg aac atg ttg ggc ggg gga ggc agt gct ggc      645
His Val Asp Ser Thr Met Asn Met Leu Gly Gly Gly Gly Ser Ala Gly
            165                 170                 175

cgg aag ccc ctc aag tcg ggt atg aag gag ctg gcc gtg ttc cgg gag      693
Arg Lys Pro Leu Lys Ser Gly Met Lys Glu Leu Ala Val Phe Arg Glu
        180                 185                 190

aag gtc act gag cag cac cgg cag atg ggc aag ggt ggc aag cat cac      741
Lys Val Thr Glu Gln His Arg Gln Met Gly Lys Gly Gly Lys His His
    195                 200                 205

ctt ggc ctg gag gag ccc aag aag ctg cga cca ccc cct gcc agg act      789
Leu Gly Leu Glu Glu Pro Lys Lys Leu Arg Pro Pro Pro Ala Arg Thr
210                 215                 220                 225

ccc tgc caa cag gaa ctg gac cag gtc ctg gag cgg atc tcc acc atg      837
Pro Cys Gln Gln Glu Leu Asp Gln Val Leu Glu Arg Ile Ser Thr Met
                230                 235                 240

cgc ctt ccg gat gag cgg ggc cct ctg gag cac ctc tac tcc ctg cac      885
Arg Leu Pro Asp Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His
            245                 250                 255

atc ccc aac tgt gac aag cat ggc ctg tac aac ctc aaa cag tgc aag      933
Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys
        260                 265                 270

atg tct ctg aac ggg cag cgt ggg gag tgc tgg tgt gtg aac ccc aac      981
Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn
    275                 280                 285

acc ggg aag ctg atc cag gga gcc ccc acc atc cgg ggg gac ccc gag     1029
Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu
290                 295                 300                 305

tgt cat ctc ttc tac aat gag cag cag gag gct cgc ggg gtg cac acc     1077
Cys His Leu Phe Tyr Asn Glu Gln Gln Glu Ala Arg Gly Val His Thr
                310                 315                 320

cag cgg atg cag tag accgcagcca gccggtgcct ggcgcccctg ccccccgccc     1132
Gln Arg Met Gln
            325

ctctccaaac accggcagaa aacggagagt gcttgggtgg tgggtgctgg aggattttcc   1192

agttctgaca cacgtattta tatttggaaa gagaccagca ccgagctcgg cacctccccg   1252

gcctctctct tcccagctgc agatgccaca cctgctcctt cttgctttcc ccgggggagg   1312

aaggggttg tggtcgggga gctggggtac aggtttgggg agggggaaga gaaattttta    1372

tttttgaacc cctgtgtccc ttttgcataa gattaaagga aggaaaagt               1421
```

<210> 4
<211> 325
<212> PRT
<213> homo sapiens

<400> 4

Met Leu Pro Arg Val Gly Cys Pro Ala Leu Pro Leu Pro Pro Pro Pro
1 5 10 15

Leu Leu Pro Leu Leu Leu Leu Leu Leu Gly Ala Ser Gly Gly Gly Gly
20 25 30

Gly Ala Arg Ala Glu Val Leu Phe Arg Cys Pro Pro Cys Thr Pro Glu
35 40 45

Arg Leu Ala Ala Cys Gly Pro Pro Pro Val Ala Pro Pro Ala Ala Val
50 55 60

Ala Ala Val Ala Gly Gly Ala Arg Met Pro Cys Ala Glu Leu Val Arg
65 70 75 80

Glu Pro Gly Cys Gly Cys Cys Ser Val Cys Ala Arg Leu Glu Gly Glu
85 90 95

Ala Cys Gly Val Tyr Thr Pro Arg Cys Gly Gln Gly Leu Arg Cys Tyr
100 105 110

Pro His Pro Gly Ser Glu Leu Pro Leu Gln Ala Leu Val Met Gly Glu
115 120 125

Gly Thr Cys Glu Lys Arg Arg Asp Ala Glu Tyr Gly Ala Ser Pro Glu
130 135 140

Gln Val Ala Asp Asn Gly Asp Asp His Ser Glu Gly Gly Leu Val Glu
145 150 155 160

Asn His Val Asp Ser Thr Met Asn Met Leu Gly Gly Gly Gly Ser Ala
165 170 175

Gly Arg Lys Pro Leu Lys Ser Gly Met Lys Glu Leu Ala Val Phe Arg
180 185 190

Glu Lys Val Thr Glu Gln His Arg Gln Met Gly Lys Gly Gly Lys His
195 200 205

His Leu Gly Leu Glu Glu Pro Lys Lys Leu Arg Pro Pro Pro Ala Arg
210 215 220

Thr Pro Cys Gln Gln Glu Leu Asp Gln Val Leu Glu Arg Ile Ser Thr
225 230 235 240

Met Arg Leu Pro Asp Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu

245 250 255

His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys
260 265 270

Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro
275 280 285

Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro
290 295 300

Glu Cys His Leu Phe Tyr Asn Glu Gln Gln Glu Ala Arg Gly Val His
305 310 315 320

Thr Gln Arg Met Gln
325

<210> 5
<211> 2502
<212> DNA
<213> homo sapiens

<220>
<221> CDS
<222> (76)..(624)

<400> 5
ctctttggcc aagccctgcc tctgtacagc ctcgagtgga cagccagagg ctgcagctgg 60

agcccagagc ccaag atg gag ccc cag ctg ggg cct gag gct gcc gcc ctc 111
Met Glu Pro Gln Leu Gly Pro Glu Ala Ala Ala Leu
1 5 10

cgc cct ggc tgg ctg gcc ctg ctg ctg tgg gtc tca gcc ctg agc tgt 159
Arg Pro Gly Trp Leu Ala Leu Leu Leu Trp Val Ser Ala Leu Ser Cys
15 20 25

tct ttc tcc ttg cca gct tct tcc ctt tct tct ctg gtg ccc caa gtc 207
Ser Phe Ser Leu Pro Ala Ser Ser Leu Ser Ser Leu Val Pro Gln Val
30 35 40

aga acc agc tac aat ttt gga agg act ttc ctc ggt ctt gat aaa tgc 255
Arg Thr Ser Tyr Asn Phe Gly Arg Thr Phe Leu Gly Leu Asp Lys Cys
45 50 55 60

aat gcc tgc atc ggg aca tct att tgc aag aag ttc ttt aaa gaa gaa 303
Asn Ala Cys Ile Gly Thr Ser Ile Cys Lys Lys Phe Phe Lys Glu Glu
65 70 75

ata aga tct gac aac tgg ctg gct tcc cac ctt gga ctg cct ccc gat 351
Ile Arg Ser Asp Asn Trp Leu Ala Ser His Leu Gly Leu Pro Pro Asp
80 85 90

tcc ttg ctt tct tat cct gca aat tac tca gat gat tcc aaa atc tgg 399
Ser Leu Leu Ser Tyr Pro Ala Asn Tyr Ser Asp Asp Ser Lys Ile Trp
95 100 105

```
cgc cct gtg gag atc ttt aga ctg gtc agc aaa tat caa aac gag atc      447
Arg Pro Val Glu Ile Phe Arg Leu Val Ser Lys Tyr Gln Asn Glu Ile
    110                 115                 120

tca gac agg aaa atc tgt gcc tct gca tca gcc cca aag acc tgc agc      495
Ser Asp Arg Lys Ile Cys Ala Ser Ala Ser Ala Pro Lys Thr Cys Ser
125                 130                 135                 140

att gag cgt gtc ctg cgg aaa aca gag agg ttc cag aaa tgg ctg cag      543
Ile Glu Arg Val Leu Arg Lys Thr Glu Arg Phe Gln Lys Trp Leu Gln
                145                 150                 155

gcc aag cgc ctc acg ccg gac ctg gtg cag gac tgt cac cag ggc cag      591
Ala Lys Arg Leu Thr Pro Asp Leu Val Gln Asp Cys His Gln Gly Gln
            160                 165                 170

aga gaa cta aag ttc ctg tgt atg ctg aga taa caccagtgaa aaagcctggc    644
Arg Glu Leu Lys Phe Leu Cys Met Leu Arg
        175                 180

atggagccca gcactgagaa cttccagaaa gtgttagcct tctcccaact gtgttatacc    704

aaccacattt tcaaatagta atcattaaag aggcttctgc atcaaacctt cacatgcagc    764

tcccatgcca ccctccagaa ttcaccaaca cacaggccca ccagcaacag gctacctttg    824

cacaatattc tctgatgaca actccaaagc cccggctctt tccaccacac tgtggtcccc    884

tagatggggc tgttgctgag cccaccccaa tccagatgtg atccccctgt gatctacttc    944

tggcaagatt ctcagtctgg acaggtcttc cctatgagat agaacctgat aaggagctag   1004

ggcaattctg acaacattac caaaggccca cataacttct aaattttggt ctggtctgaa   1064

ggaaaacctg ttctcgccct agtgatggat gaactctctt atctctggct tctagaggga   1124

aaaaaaaagc atacctcttt tacttttttaa gtacctccat cagagtcatg aaatcacctg   1184

tcaagactat ctatctttta tgtttccatt ctggtaagaa ctctttaaat gaggacactg   1244

ctgattgctg gtgatgtttt ttgagcaaac actcgggggt atggatgaaa gccaatcgca   1304

ggtcaaatga ctccttgggg aagctacttc tcctctattc agatttcact aaaatcttcc   1364

aagatgaaag caaatctaga tttcggtctt cattgctgtc cattttgta atgaacgagt   1424

gtttttcctt tagctagtgt atcaggcagg gttctaccag agaaacagaa ccagtaggag   1484

atacatatac atgtccagat ttatttcaaa gaattgattt acatgattgt ggggattggc   1544

aagtccaaaa tccatatggt aggcctgcaa tctgtaaacc tttgggcagg agctgatgct   1604

gtagtttgca gatagaattc cttgttcctt aaaaaaatct gtttttgttc ttaagggctt   1664

tgaatgattg gatcaggccc acccagatta cctagataat ctcttttact taaagtaaac   1724

tgattgtagg tgctaatcac atctatgaaa tgccttcaca gcaacaccta gattagcatt   1784

caattgaata actggggaat acagcctagc caagttgaca cataaaatta accatcacag   1844

caacatgcct gctaaatttt atcgaccgtc ttcagactgt taaggattgt ggtagagaac   1904
```

tgtgacagcc actctcagca tcaccctgaa ccaaaggccc ctatcaagta acaatatagc 1964

caagcaaaat tccagtcaat agagacattg actggttggc tggcttccca agggatagca 2024

ccagacaaga aatgcaagga tgaggaaacc aggcacggga gagggagggg caacagaggt 2084

ccagggtttg gttatctttt tatttttcac tgggaggtgg taagttagcc ctgttgccca 2144

tgtatgcaga tgggagaagt gatttagaaa ctccaaagca attggtaatc cccaaaatgg 2204

gtgtatctgg tttgaaatga aaccttattt tattggaaat ggttggtttc ccaattctgt 2264

ttgccattgg ccaatataat tgtgggtttg cacatggcca gcacatgcca aacagaagta 2324

gacaaaggtc tcactctgta agtgggacct tggggaggag ctgcctccat cataaaggga 2384

ggggttagta aaaatggtct cttaagcctg ttcctgctac agttatagag gttgctcaga 2444

accttctcag caaatatagc agttatctat tgttgtgtat taaaccattt caacacat 2502

<210> 6
<211> 182
<212> PRT
<213> homo sapiens

<400> 6

Met Glu Pro Gln Leu Gly Pro Glu Ala Ala Ala Leu Arg Pro Gly Trp
1 5 10 15

Leu Ala Leu Leu Leu Trp Val Ser Ala Leu Ser Cys Ser Phe Ser Leu
20 25 30

Pro Ala Ser Ser Leu Ser Ser Leu Val Pro Gln Val Arg Thr Ser Tyr
35 40 45

Asn Phe Gly Arg Thr Phe Leu Gly Leu Asp Lys Cys Asn Ala Cys Ile
50 55 60

Gly Thr Ser Ile Cys Lys Lys Phe Phe Lys Glu Glu Ile Arg Ser Asp
65 70 75 80

Asn Trp Leu Ala Ser His Leu Gly Leu Pro Pro Asp Ser Leu Leu Ser
85 90 95

Tyr Pro Ala Asn Tyr Ser Asp Asp Ser Lys Ile Trp Arg Pro Val Glu
100 105 110

Ile Phe Arg Leu Val Ser Lys Tyr Gln Asn Glu Ile Ser Asp Arg Lys
115 120 125

Ile Cys Ala Ser Ala Ser Ala Pro Lys Thr Cys Ser Ile Glu Arg Val
130 135 140

Leu Arg Lys Thr Glu Arg Phe Gln Lys Trp Leu Gln Ala Lys Arg Leu
145 150 155 160

Thr Pro Asp Leu Val Gln Asp Cys His Gln Gly Gln Arg Glu Leu Lys
165 170 175

Phe Leu Cys Met Leu Arg
180

<210> 7
<211> 5668
<212> DNA
<213> homo sapiens

<220>
<221> CDS
<222> (163)..(2475)

<400> 7
gctttgtttg atggtgatcc acatttatcc acagagaatc ctgccttggt tcctgatgct 60

ttgctagcct cagacacttg tctggatata agcgaagctg cctttgacca cagtttcagc 120

gatgcctcag gtctcaacac atccacggga acaatagatg ac atg agt aaa ctg 174
Met Ser Lys Leu
1

aca tta tcc gaa ggc cat ccg gaa acg cca gtt gat ggg gac cta ggg 222
Thr Leu Ser Glu Gly His Pro Glu Thr Pro Val Asp Gly Asp Leu Gly
5 10 15 20

aag caa gat atc tgc tca tct gaa gcc tcg tgg ggt gat ttt gaa tat 270
Lys Gln Asp Ile Cys Ser Ser Glu Ala Ser Trp Gly Asp Phe Glu Tyr
25 30 35

gat gta atg ggc cag aat atc gat gaa gat tta ctg aga gag cct gaa 318
Asp Val Met Gly Gln Asn Ile Asp Glu Asp Leu Leu Arg Glu Pro Glu
40 45 50

cac ttc ctg tat ggt ggt gac cct cct ttg gag gaa gat tct ctg aag 366
His Phe Leu Tyr Gly Gly Asp Pro Pro Leu Glu Glu Asp Ser Leu Lys
55 60 65

cag tcg ctg gca ccg tac aca cct ccc ttt gat ttg tct tat ctc aca 414
Gln Ser Leu Ala Pro Tyr Thr Pro Pro Phe Asp Leu Ser Tyr Leu Thr
70 75 80

gaa cct gcc cag agt gct gaa aca ata gag gaa gct ggg tct cca gag 462
Glu Pro Ala Gln Ser Ala Glu Thr Ile Glu Glu Ala Gly Ser Pro Glu
85 90 95 100

gat gaa tct ctg gga tgc aga gca gca gag ata gtg ctt tct gca ctt 510
Asp Glu Ser Leu Gly Cys Arg Ala Ala Glu Ile Val Leu Ser Ala Leu
105 110 115

cct gat cga aga agt gag gga aac cag gct gag acc aaa aac aga ctg 558
Pro Asp Arg Arg Ser Glu Gly Asn Gln Ala Glu Thr Lys Asn Arg Leu
120 125 130

```
cct gga tcc cag ctg gct gtg ctg cat att cgt gaa gac cct gag tcc      606
Pro Gly Ser Gln Leu Ala Val Leu His Ile Arg Glu Asp Pro Glu Ser
        135                 140                 145

gtt tat ttg ccg gta gga gca ggc tcc aac att ttg tct cca tca aac      654
Val Tyr Leu Pro Val Gly Ala Gly Ser Asn Ile Leu Ser Pro Ser Asn
    150                 155                 160

gtt gac tgg gaa gta gaa aca gat aat tct gat tta cca gca ggt gga      702
Val Asp Trp Glu Val Glu Thr Asp Asn Ser Asp Leu Pro Ala Gly Gly
165                 170                 175                 180

gac ata gga cca cca aat ggt gcc agc aag gaa ata tca gaa ttg gaa      750
Asp Ile Gly Pro Pro Asn Gly Ala Ser Lys Glu Ile Ser Glu Leu Glu
                185                 190                 195

gaa gaa aaa aca att cct acc aaa gag cct gag cag ata aaa tca gaa      798
Glu Glu Lys Thr Ile Pro Thr Lys Glu Pro Glu Gln Ile Lys Ser Glu
            200                 205                 210

tac aag gaa gaa aga tgt aca gag aag aat gaa gat cgt cat gca cta      846
Tyr Lys Glu Glu Arg Cys Thr Glu Lys Asn Glu Asp Arg His Ala Leu
        215                 220                 225

cac atg gat tac ata ctt gta aac cgt gaa gaa aat tca cac tca aag      894
His Met Asp Tyr Ile Leu Val Asn Arg Glu Glu Asn Ser His Ser Lys
    230                 235                 240

cca gag acc tgt gaa gaa aga gaa agc ata gct gaa tta gaa ttg tat      942
Pro Glu Thr Cys Glu Glu Arg Glu Ser Ile Ala Glu Leu Glu Leu Tyr
245                 250                 255                 260

gta ggt tcc aaa gaa aca ggg ctg cag gga act cag tta gca agc ttc      990
Val Gly Ser Lys Glu Thr Gly Leu Gln Gly Thr Gln Leu Ala Ser Phe
                265                 270                 275

cca gac aca tgt cag cca gcc tcc tta aat gaa aga aaa ggt ctc tct     1038
Pro Asp Thr Cys Gln Pro Ala Ser Leu Asn Glu Arg Lys Gly Leu Ser
            280                 285                 290

gca gag aaa atg tct tct aaa agc gat acg aga tca tct ttt gaa agc     1086
Ala Glu Lys Met Ser Ser Lys Ser Asp Thr Arg Ser Ser Phe Glu Ser
        295                 300                 305

cct gca caa gac cag agt tgg atg ttc ttg ggc cat agt gag gtt ggt     1134
Pro Ala Gln Asp Gln Ser Trp Met Phe Leu Gly His Ser Glu Val Gly
    310                 315                 320

gat cca tca ctg gat gcc agg gac tca ggg cct ggg tgg tct ggc aag     1182
Asp Pro Ser Leu Asp Ala Arg Asp Ser Gly Pro Gly Trp Ser Gly Lys
325                 330                 335                 340

act gtg gag ccg ttc tct gaa ctc ggc ttg ggt gag ggt ccc cag ctg     1230
Thr Val Glu Pro Phe Ser Glu Leu Gly Leu Gly Glu Gly Pro Gln Leu
                345                 350                 355

cag att ctg gaa gaa atg aag cct cta gaa tct ttg gca cta gag gaa     1278
Gln Ile Leu Glu Glu Met Lys Pro Leu Glu Ser Leu Ala Leu Glu Glu
            360                 365                 370

gcc tct ggt cca gtc agc caa tca cag aag agt aag agc cga ggc agg     1326
Ala Ser Gly Pro Val Ser Gln Ser Gln Lys Ser Lys Ser Arg Gly Arg
```

375 380 385

gct ggc ccg gat gca gtt acg ttg cag gct gtc acc cat gac aat gaa 1374
Ala Gly Pro Asp Ala Val Thr Leu Gln Ala Val Thr His Asp Asn Glu
390 395 400

tgg gaa atg ctt tca cca cag cct gtt cag aaa aac atg atc cct gac 1422
Trp Glu Met Leu Ser Pro Gln Pro Val Gln Lys Asn Met Ile Pro Asp
405 410 415 420

acg gaa atg gag gag gag aca gag ttc ctt gag ctc gga acc agg ata 1470
Thr Glu Met Glu Glu Glu Thr Glu Phe Leu Glu Leu Gly Thr Arg Ile
425 430 435

tca aga cca aat gga cta ctg tca gag gat gta gga atg gac atc ccc 1518
Ser Arg Pro Asn Gly Leu Leu Ser Glu Asp Val Gly Met Asp Ile Pro
440 445 450

ttt gaa gag ggc gtg ctg agt ccc agt gct gca gac atg agg cct gaa 1566
Phe Glu Glu Gly Val Leu Ser Pro Ser Ala Ala Asp Met Arg Pro Glu
455 460 465

cct cct aat tct ctg gat ctt aat gac act cat cct cgg aga atc aag 1614
Pro Pro Asn Ser Leu Asp Leu Asn Asp Thr His Pro Arg Arg Ile Lys
470 475 480

ctc aca gcc cca aat atc aat ctt tct ctg gac caa agt gaa gga tct 1662
Leu Thr Ala Pro Asn Ile Asn Leu Ser Leu Asp Gln Ser Glu Gly Ser
485 490 495 500

att ctc tct gat gat aac ttg gac agt cca gat gaa att gac atc aat 1710
Ile Leu Ser Asp Asp Asn Leu Asp Ser Pro Asp Glu Ile Asp Ile Asn
505 510 515

gtg gat gaa ctt gat acc ccc gat gaa gca gat tct ttt gag tac act 1758
Val Asp Glu Leu Asp Thr Pro Asp Glu Ala Asp Ser Phe Glu Tyr Thr
520 525 530

ggc cat gat ccc aca gcc aac aaa gat tct ggc caa gag tca gag tct 1806
Gly His Asp Pro Thr Ala Asn Lys Asp Ser Gly Gln Glu Ser Glu Ser
535 540 545

att cca gaa tat acg gcc gaa gag gaa cgg gag gac aac cgg ctt tgg 1854
Ile Pro Glu Tyr Thr Ala Glu Glu Glu Arg Glu Asp Asn Arg Leu Trp
550 555 560

agg aca gtg gtc att gga gaa caa gag cag cgc att gac atg aag gtc 1902
Arg Thr Val Val Ile Gly Glu Gln Glu Gln Arg Ile Asp Met Lys Val
565 570 575 580

atc gag ccc tac agg aga gtc att tct cac gga gga tac tat ggg gac 1950
Ile Glu Pro Tyr Arg Arg Val Ile Ser His Gly Gly Tyr Tyr Gly Asp
585 590 595

ggt cta aat gcc atc att gtg ttt gcc gcc tgt ttt ctg cca gac agc 1998
Gly Leu Asn Ala Ile Ile Val Phe Ala Ala Cys Phe Leu Pro Asp Ser
600 605 610

agt cgg gcg gat tac cac tat gtc atg gaa aat ctt ttc cta tat gta 2046
Ser Arg Ala Asp Tyr His Tyr Val Met Glu Asn Leu Phe Leu Tyr Val
615 620 625

ata agt act tta gag ttg atg gta gct gaa gac tat atg att gtg tac 2094

```
Ile Ser Thr Leu Glu Leu Met Val Ala Glu Asp Tyr Met Ile Val Tyr
    630                 635                 640

ttg aat ggt gca acc cca aga agg agg atg cca ggg cta ggc tgg atg      2142
Leu Asn Gly Ala Thr Pro Arg Arg Arg Met Pro Gly Leu Gly Trp Met
645                 650                 655                 660

aag aaa tgc tac cag atg att gac aga cgg ttg agg aag aat ttg aaa      2190
Lys Lys Cys Tyr Gln Met Ile Asp Arg Arg Leu Arg Lys Asn Leu Lys
                665                 670                 675

tca ttc atc att gtt cat cca tct tgg ttc atc aga aca atc ctt gct      2238
Ser Phe Ile Ile Val His Pro Ser Trp Phe Ile Arg Thr Ile Leu Ala
            680                 685                 690

gtg aca cga cct ttt ata agt tca aaa ttc agc agt aaa att aaa tat      2286
Val Thr Arg Pro Phe Ile Ser Ser Lys Phe Ser Ser Lys Ile Lys Tyr
        695                 700                 705

gtc aat agc tta tca gaa ctc agt ggg ctg atc cca atg gat tgc atc      2334
Val Asn Ser Leu Ser Glu Leu Ser Gly Leu Ile Pro Met Asp Cys Ile
    710                 715                 720

cac att cca gag agc atc atc aaa ctg gat gaa gaa ctg agg gaa gca      2382
His Ile Pro Glu Ser Ile Ile Lys Leu Asp Glu Glu Leu Arg Glu Ala
725                 730                 735                 740

tca gag gca gct aaa act agc tgc ctt tac aat gat cca gaa atg tct      2430
Ser Glu Ala Ala Lys Thr Ser Cys Leu Tyr Asn Asp Pro Glu Met Ser
                745                 750                 755

tct atg gag aag gat att gac ttg aag ctg aaa gaa aag cct tag          2475
Ser Met Glu Lys Asp Ile Asp Leu Lys Leu Lys Glu Lys Pro
            760                 765                 770

ttggccatgc tggaagaaga ggatgctttt ctggttcatg gttctgttga aacatatcta   2535

cctgaaagag acagggctga tgttaccttt ttccactttg cactacctgg tgccattcta   2595

aatttctaag gggaaaaata gaaagtttgt ttactcttaa gatattttat gaaattgtgt   2655

gtactttcct attttgccaa ttatgtgcct caaagatttt agttgagcct tagcaagaaa   2715

gtaggacctt ccatttcaat acttcattaa cacggtgtag tgatactttg tcccttagac   2775

tggtgtttac cagtaagata cctttaatcc actgttaagt atgagtggat ttgtttccat   2835

agattagctg gatttccttt tggtgattgc attaggttta aagtacacag gtctcaactc   2895

tccccaggaa agtttcccct gtttgactcc acctttaaaa tcctaagcct gactaggaca   2955

gccacaaacc acacaaggtg taaaaccatc atcagctaag tgcccgtttt gttcttgttt   3015

accagaatct cctttaactt ctcaaaggga agccgggctt tctaatccac gtcaacttta   3075

ttttagttgt caaattgggc attatatttt atgtaaattg gtcttttaac atcattttcc   3135

tgatgaatgt tggtgaccac cacattgtga aatttaagaa tccgtgttgc atgtttggta   3195

gctctctgag tttcaggcca taaactcagc tccagaggtt acctttaag tgccaagaac   3255

tcaagtgcaa ggtggcctac tcaaaaatca tttggtagca ttcagttatt catgaattcc   3315
```

tctctcgcat gcattataaa aagtgatctg ctttaaaaca ccgtaatctg atcataggct 3375

taaaattaaa tatgagtatt actttcatgt acaaaatatt tcctttatag tcttcatatg 3435

ccctttaaaa tgccaacaag atttcaagtc tgtaggcctc tagtgaggtg gggtggcaaa 3495

ccacagctaa gtctcgctca ccactgcaag ctaagaatgg tttttacatt ttgggttgga 3555

aaaatttttt ttgaatattt catgacacat gaaaattatt caaatgttag tgccgataaa 3615

taaagtggta ctgaaacaca gccacacaaa cttgtttttg tactgtctac agctactttc 3675

acactacagc cgcagagctg agcagttcag cagaccgtat gtcccacaat gcctaaaaca 3735

ttgactatgt ttacagaaaa agtttgctga cccctgctct agcaaacgca tcctttccta 3795

ctccacccca atttgtattt agatagtttc tctaacagaa cggacaaatg aggctgcaaa 3855

ctaatttatt tttgtcaaaa atcaatgttt tgacatccac agacagtgaa ataaaagaaa 3915

tggcttgctg aaaaacatga ggagtcctag ccacaaaatc actgcttagg ttgcaattgc 3975

caaaatgaag ccttcttaga agcacttctt tagtatatac aggtgttggc tgaagtccgt 4035

gcctcactct gggaaccatt cttagtctcc agtgtctcct attacaaaga agctggcaga 4095

aataaaaatg aaggggtgag agcggttcca ccctagtctc atggtggaaa attcattggg 4155

gagagctgtc caggatattt ggagtcctgg gtagaaggag cttgtaacta ctttaaagtc 4215

gacatctttg cacaggtgat tgagtttctc tgacctcatt gcttcacctc tgtctcctcc 4275

cgtccttccg cacgtgccca cacacacgca gttcagccct ctttcctcca taagcctcca 4335

tcgttttctc ttttctcctc ttgatccttt caagcgagta tcttgttgaa ttgtatgttc 4395

tgttggatct cctccttcat aacatctggc ttgttggaca gaaaaaccct acagcccacc 4455

ccctcccaca gcccacctcc acttttgaaa gcccaaatta cacctctccc agaacacagt 4515

gttgacgtaa atacagttac ccaatattcc tgtttgttca cctatttgct actttcactc 4575

agtagcatcc cattttgtaa aatgaattcc atggtcaccc tgtcacagga agtaatgaaa 4635

aatccagtgt tcagtgtagt ggtgcaaacc tgagggcata gagctgttca tagagggctc 4695

ttgttatagc caaacagaca cagcaacaat ctcaccattt atatatatat ttttaacttg 4755

tccagctcat ctatggaaaa ctactcaggt ggtatgctgt ttgaagcctc atcttcctac 4815

atgaaaatta tgggcatttg tcccaatgat tttgtttcag ctgttctgta ggctgcataa 4875

ccactctgat atttaggtat ctgctatttt attatcttaa aagacaaatt aatttaattg 4935

catgtgctag ggaaaagcta ccatgtacat tcaccccaag taaatagaat cctagatgaa 4995

tcctagaaaa ataatcccta agcagatagg tagacagagg taaacattca catgatttag 5055

ctctctagct cttgcactct gaacattctt gctttggttc tgacttctgg gaactgcttt 5115

gcatttctcc tatagatctg tagttaaggg aaccaagggg tcattggggc aaaagcattg 5175

tttctcaaag ctccttgatt aagagaaaga acagaaattt gcacagaaga tagtgtcaag 5235

gagtgagaaa gtttgtttga gggcagtagc tcagtgtgga agaaaatcct gaagtttctg 5295

ttgaagccat acaatgttct atggggttac tctctaagac attctctgag gtgtgtgagg 5355

aagtcactac tcctagcctt tgttaagatg taattttaaa tattcagtta tggtactatg 5415

tttgcaactc tcgtcttatc acaatgcctc agtagtttgt tcccttagaa acatttagat 5475

gtgcacaaat taatctttta tatatctaaa ggtttttcta tcatgcattg gattgctcag 5535

aataaagtgt ctgttagact tcgttttggt aaataaattc tccataatgt agattaataa 5595

tataaaagtc tttaatgaca caatatatct atatagcctc actgtataat tcagaaataa 5655

aaattgattc tgc 5668

<210> 8
<211> 770
<212> PRT
<213> homo sapiens

<400> 8

Met Ser Lys Leu Thr Leu Ser Glu Gly His Pro Glu Thr Pro Val Asp
1 5 10 15

Gly Asp Leu Gly Lys Gln Asp Ile Cys Ser Ser Glu Ala Ser Trp Gly
20 25 30

Asp Phe Glu Tyr Asp Val Met Gly Gln Asn Ile Asp Glu Asp Leu Leu
35 40 45

Arg Glu Pro Glu His Phe Leu Tyr Gly Gly Asp Pro Pro Leu Glu Glu
50 55 60

Asp Ser Leu Lys Gln Ser Leu Ala Pro Tyr Thr Pro Pro Phe Asp Leu
65 70 75 80

Ser Tyr Leu Thr Glu Pro Ala Gln Ser Ala Glu Thr Ile Glu Glu Ala
85 90 95

Gly Ser Pro Glu Asp Glu Ser Leu Gly Cys Arg Ala Ala Glu Ile Val
100 105 110

Leu Ser Ala Leu Pro Asp Arg Arg Ser Glu Gly Asn Gln Ala Glu Thr
115 120 125

Lys Asn Arg Leu Pro Gly Ser Gln Leu Ala Val Leu His Ile Arg Glu
130 135 140

Asp Pro Glu Ser Val Tyr Leu Pro Val Gly Ala Gly Ser Asn Ile Leu
145 150 155 160

Ser Pro Ser Asn Val Asp Trp Glu Val Glu Thr Asp Asn Ser Asp Leu
165 170 175

Pro Ala Gly Gly Asp Ile Gly Pro Pro Asn Gly Ala Ser Lys Glu Ile
180 185 190

Ser Glu Leu Glu Glu Glu Lys Thr Ile Pro Thr Lys Glu Pro Glu Gln
195 200 205

Ile Lys Ser Glu Tyr Lys Glu Glu Arg Cys Thr Glu Lys Asn Glu Asp
210 215 220

Arg His Ala Leu His Met Asp Tyr Ile Leu Val Asn Arg Glu Glu Asn
225 230 235 240

Ser His Ser Lys Pro Glu Thr Cys Glu Glu Arg Glu Ser Ile Ala Glu
245 250 255

Leu Glu Leu Tyr Val Gly Ser Lys Glu Thr Gly Leu Gln Gly Thr Gln
260 265 270

Leu Ala Ser Phe Pro Asp Thr Cys Gln Pro Ala Ser Leu Asn Glu Arg
275 280 285

Lys Gly Leu Ser Ala Glu Lys Met Ser Ser Lys Ser Asp Thr Arg Ser
290 295 300

Ser Phe Glu Ser Pro Ala Gln Asp Gln Ser Trp Met Phe Leu Gly His
305 310 315 320

Ser Glu Val Gly Asp Pro Ser Leu Asp Ala Arg Asp Ser Gly Pro Gly
325 330 335

Trp Ser Gly Lys Thr Val Glu Pro Phe Ser Glu Leu Gly Leu Gly Glu
340 345 350

Gly Pro Gln Leu Gln Ile Leu Glu Glu Met Lys Pro Leu Glu Ser Leu
355 360 365

Ala Leu Glu Glu Ala Ser Gly Pro Val Ser Gln Ser Gln Lys Ser Lys
370 375 380

Ser Arg Gly Arg Ala Gly Pro Asp Ala Val Thr Leu Gln Ala Val Thr
385 390 395 400

His Asp Asn Glu Trp Glu Met Leu Ser Pro Gln Pro Val Gln Lys Asn

405 410 415

Met Ile Pro Asp Thr Glu Met Glu Glu Glu Thr Glu Phe Leu Glu Leu
420 425 430

Gly Thr Arg Ile Ser Arg Pro Asn Gly Leu Leu Ser Glu Asp Val Gly
435 440 445

Met Asp Ile Pro Phe Glu Glu Gly Val Leu Ser Pro Ser Ala Ala Asp
450 455 460

Met Arg Pro Glu Pro Pro Asn Ser Leu Asp Leu Asn Asp Thr His Pro
465 470 475 480

Arg Arg Ile Lys Leu Thr Ala Pro Asn Ile Asn Leu Ser Leu Asp Gln
485 490 495

Ser Glu Gly Ser Ile Leu Ser Asp Asp Asn Leu Asp Ser Pro Asp Glu
500 505 510

Ile Asp Ile Asn Val Asp Glu Leu Asp Thr Pro Asp Glu Ala Asp Ser
515 520 525

Phe Glu Tyr Thr Gly His Asp Pro Thr Ala Asn Lys Asp Ser Gly Gln
530 535 540

Glu Ser Glu Ser Ile Pro Glu Tyr Thr Ala Glu Glu Glu Arg Glu Asp
545 550 555 560

Asn Arg Leu Trp Arg Thr Val Val Ile Gly Glu Gln Glu Gln Arg Ile
565 570 575

Asp Met Lys Val Ile Glu Pro Tyr Arg Arg Val Ile Ser His Gly Gly
580 585 590

Tyr Tyr Gly Asp Gly Leu Asn Ala Ile Ile Val Phe Ala Ala Cys Phe
595 600 605

Leu Pro Asp Ser Ser Arg Ala Asp Tyr His Tyr Val Met Glu Asn Leu
610 615 620

Phe Leu Tyr Val Ile Ser Thr Leu Glu Leu Met Val Ala Glu Asp Tyr
625 630 635 640

Met Ile Val Tyr Leu Asn Gly Ala Thr Pro Arg Arg Arg Met Pro Gly
645 650 655

Leu Gly Trp Met Lys Lys Cys Tyr Gln Met Ile Asp Arg Arg Leu Arg
660 665 670

Lys Asn Leu Lys Ser Phe Ile Ile Val His Pro Ser Trp Phe Ile Arg
675 680 685

Thr Ile Leu Ala Val Thr Arg Pro Phe Ile Ser Ser Lys Phe Ser Ser
690 695 700

Lys Ile Lys Tyr Val Asn Ser Leu Ser Glu Leu Ser Gly Leu Ile Pro
705 710 715 720

Met Asp Cys Ile His Ile Pro Glu Ser Ile Ile Lys Leu Asp Glu Glu
725 730 735

Leu Arg Glu Ala Ser Glu Ala Ala Lys Thr Ser Cys Leu Tyr Asn Asp
740 745 750

Pro Glu Met Ser Ser Met Glu Lys Asp Ile Asp Leu Lys Leu Lys Glu
755 760 765

Lys Pro
770

<210> 9
<211> 1589
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (169)..(597)

<400> 9
gtctcttcct cgctccctct ctttctctcc tccctctgcc ttcccagtgc ataaagtctc 60

tgtcgctccc ggaacttgtt ggcaatgcct atttttggc tttccccgc gttctctaaa 120

ctaactattt aaaggtctgc ggtcgcaaat ggtttgacta aacgtagg atg gga ctt 177
Met Gly Leu
1

aag ttg aac ggc aga tat att tca ctg atc ctc gcg gtg caa ata gcg 225
Lys Leu Asn Gly Arg Tyr Ile Ser Leu Ile Leu Ala Val Gln Ile Ala
5 10 15

tat ctg gtg cag gcc gtg aga gca gcg ggc aag tgc gat gcg gtc ttc 273
Tyr Leu Val Gln Ala Val Arg Ala Ala Gly Lys Cys Asp Ala Val Phe
20 25 30 35

aag ggc ttt tcg gac tgt ttg ctc aag ctg ggc gac agc atg gcc aac 321
Lys Gly Phe Ser Asp Cys Leu Leu Lys Leu Gly Asp Ser Met Ala Asn
40 45 50

```
tac ccg cag ggc ctg gac gac aag acg aac atc aag acc gtg tgc aca      369
Tyr Pro Gln Gly Leu Asp Asp Lys Thr Asn Ile Lys Thr Val Cys Thr
            55                  60                  65

tac tgg gag gat ttc cac agc tgc acg gtc aca gcc ctt acg gat tgc      417
Tyr Trp Glu Asp Phe His Ser Cys Thr Val Thr Ala Leu Thr Asp Cys
        70                  75                  80

cag gaa ggg gcg aaa gat atg tgg gat aaa ctg aga aaa gaa tcc aaa      465
Gln Glu Gly Ala Lys Asp Met Trp Asp Lys Leu Arg Lys Glu Ser Lys
    85                  90                  95

aac ctc aac atc caa ggc agc tta ttc gaa ctc tgc ggc agc ggc aac      513
Asn Leu Asn Ile Gln Gly Ser Leu Phe Glu Leu Cys Gly Ser Gly Asn
100                 105                 110                 115

ggg gcg gcg ggg tcc ctg ctc ccg gcg ttc ccg gtg ctc ctg gtg tct      561
Gly Ala Ala Gly Ser Leu Leu Pro Ala Phe Pro Val Leu Leu Val Ser
                120                 125                 130

ctc tcg gca gct tta gcg acc tgg ctt tcc ttc tga gcgtggggcc            607
Leu Ser Ala Ala Leu Ala Thr Trp Leu Ser Phe
            135                 140

agctcccccc gcgcgcccac ccacactcac tccatgctcc cggaaatcga gaggaagatc    667

cattagttct ttggggacgt tgtgattctc tgtgatgctg aaaacactca tataggattg    727

tgggaaatcc tgattctctt ttttatttcg tttgatttct tgtgttttat ttgccaaatg    787

ttaccaatca gtgagcaagc aagcacagcc aaaatcggac ctcagcttta gtccgtcttc    847

acacacaaat aagaaaacgg caaacccacc ccatttttta attttattat tattaatttt    907

ttttgttggc aaaagaatct caggaacggc cctgggccac ctactatatt aatcatgcta    967

gtaacatgaa aaatgatggg ctcctcctaa taggaaggcg aggagaggag aaggccaggg   1027

gaatgaattc aagagagatg tccacggccg aaacatacgg tgaataattc acgctcacgt   1087

cgttcttcca cagtatcttg ttttgatcat ttccactgca catttctcct caagaaaagc   1147

gaaaggacag actgttggct ttgtgtttgg aggataggag ggagagaggg aaggggctga   1207

ggaaatctct ggggtaagag taaaggcttc cagaagacat gctgctatgg tcactgaggg   1267

gttagcttta tctgctgttg ttgatgcatc cgtccaagtt cactgccttt attttccctc   1327

ctcctcttg ttttagctgt tacacacaca gtaatacctg aatatccaac ggtatagatc   1387

acaaggggg gatgttaaat gttaatctaa aatatagcta aaaaaagatt ttgacataaa   1447

agagccttga ttttaaaaaa aaaagagaga gagatgtaat ttaaaaagtt tattataaat   1507

taaattcagc aaaaaaagat ttgctacaaa gtatagagaa gtataaaata aaagttattg   1567

tttgaaaaaa aaaaaaaaaa aa                                            1589
```

<210> 10
<211> 142
<212> PRT
<213> Homo sapiens

<400> 10

Met Gly Leu Lys Leu Asn Gly Arg Tyr Ile Ser Leu Ile Leu Ala Val
1 5 10 15

Gln Ile Ala Tyr Leu Val Gln Ala Val Arg Ala Ala Gly Lys Cys Asp
20 25 30

Ala Val Phe Lys Gly Phe Ser Asp Cys Leu Leu Lys Leu Gly Asp Ser
35 40 45

Met Ala Asn Tyr Pro Gln Gly Leu Asp Asp Lys Thr Asn Ile Lys Thr
50 55 60

Val Cys Thr Tyr Trp Glu Asp Phe His Ser Cys Thr Val Thr Ala Leu
65 70 75 80

Thr Asp Cys Gln Glu Gly Ala Lys Asp Met Trp Asp Lys Leu Arg Lys
85 90 95

Glu Ser Lys Asn Leu Asn Ile Gln Gly Ser Leu Phe Glu Leu Cys Gly
100 105 110

Ser Gly Asn Gly Ala Ala Gly Ser Leu Leu Pro Ala Phe Pro Val Leu
115 120 125

Leu Val Ser Leu Ser Ala Ala Leu Ala Thr Trp Leu Ser Phe
130 135 140

<210> 11
<211> 5180
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (49)..(4161)

<400> 11
accgggagcg cgcgctctga tccgaggaga ccccgcgctc ccgcagcc atg ggc acc 57
Met Gly Thr
1

ggg ggc cgg cgg ggg gcg gcg gcc gcg ccg ctg ctg gtg gcg gtg gcc 105
Gly Gly Arg Arg Gly Ala Ala Ala Pro Leu Leu Val Ala Val Ala
5 10 15

gcg ctg cta ctg ggc gcc gcg ggc cac ctg tac ccc gga gag gtg tgt 153
Ala Leu Leu Leu Gly Ala Ala Gly His Leu Tyr Pro Gly Glu Val Cys
20 25 30 35

ccc ggc atg gat atc cgg aac aac ctc act agg ttg cat gag ctg gag 201

```
Pro Gly Met Asp Ile Arg Asn Asn Leu Thr Arg Leu His Glu Leu Glu
                40                  45                  50

aat tgc tct gtc atc gaa gga cac ttg cag ata ctc ttg atg ttc aaa      249
Asn Cys Ser Val Ile Glu Gly His Leu Gln Ile Leu Leu Met Phe Lys
            55                  60                  65

acg agg ccc gaa gat ttc cga gac ctc agt ttc ccc aaa ctc atc atg      297
Thr Arg Pro Glu Asp Phe Arg Asp Leu Ser Phe Pro Lys Leu Ile Met
        70                  75                  80

atc act gat tac ttg ctg ctc ttc cgg gtc tat ggg ctc gag agc ctg      345
Ile Thr Asp Tyr Leu Leu Leu Phe Arg Val Tyr Gly Leu Glu Ser Leu
    85                  90                  95

aag gac ctg ttc ccc aac ctc acg gtc atc cgg gga tca cga ctg ttc      393
Lys Asp Leu Phe Pro Asn Leu Thr Val Ile Arg Gly Ser Arg Leu Phe
100                 105                 110                 115

ttt aac tac gcg ctg gtc atc ttc gag atg gtt cac ctc aag gaa ctc      441
Phe Asn Tyr Ala Leu Val Ile Phe Glu Met Val His Leu Lys Glu Leu
                120                 125                 130

ggc ctc tac aac ctg atg aac atc acc cgg ggt tct gtc cgc atc gag      489
Gly Leu Tyr Asn Leu Met Asn Ile Thr Arg Gly Ser Val Arg Ile Glu
            135                 140                 145

aag aac aat gag ctc tgt tac ttg gcc act atc gac tgg tcc cgt atc      537
Lys Asn Asn Glu Leu Cys Tyr Leu Ala Thr Ile Asp Trp Ser Arg Ile
        150                 155                 160

ctg gat tcc gtg gag gat aat tac atc gtg ttg aac aaa gat gac aac      585
Leu Asp Ser Val Glu Asp Asn Tyr Ile Val Leu Asn Lys Asp Asp Asn
    165                 170                 175

gag gag tgt gga gac atc tgt ccg ggt acc gcg aag ggc aag acc aac      633
Glu Glu Cys Gly Asp Ile Cys Pro Gly Thr Ala Lys Gly Lys Thr Asn
180                 185                 190                 195

tgc ccc gcc acc gtc atc aac ggg cag ttt gtc gaa cga tgt tgg act      681
Cys Pro Ala Thr Val Ile Asn Gly Gln Phe Val Glu Arg Cys Trp Thr
                200                 205                 210

cat agt cac tgc cag aaa gtt tgc ccg acc atc tgt aag tca cac ggc      729
His Ser His Cys Gln Lys Val Cys Pro Thr Ile Cys Lys Ser His Gly
            215                 220                 225

tgc acc gcc gaa ggc ctc tgt tgc cac agc gag tgc ctg ggc aac tgt      777
Cys Thr Ala Glu Gly Leu Cys Cys His Ser Glu Cys Leu Gly Asn Cys
        230                 235                 240

tct cag ccc gac gac ccc acc aag tgc gtg gcc tgc cgc aac ttc tac      825
Ser Gln Pro Asp Asp Pro Thr Lys Cys Val Ala Cys Arg Asn Phe Tyr
    245                 250                 255

ctg gac ggc agg tgt gtg gag acc tgc ccg ccc ccg tac tac cac ttc      873
Leu Asp Gly Arg Cys Val Glu Thr Cys Pro Pro Pro Tyr Tyr His Phe
260                 265                 270                 275

cag gac tgg cgc tgt gtg aac ttc agc ttc tgc cag gac ctg cac cac      921
Gln Asp Trp Arg Cys Val Asn Phe Ser Phe Cys Gln Asp Leu His His
                280                 285                 290
```

```
aaa tgc aag aac tcg cgg agg cag ggc tgc cac cag tac gtc att cac      969
Lys Cys Lys Asn Ser Arg Arg Gln Gly Cys His Gln Tyr Val Ile His
            295                 300                 305

aac aac aag tgc atc cct gag tgt ccc tcc ggg tac acg atg aat tcc     1017
Asn Asn Lys Cys Ile Pro Glu Cys Pro Ser Gly Tyr Thr Met Asn Ser
        310                 315                 320

agc aac ttg ctg tgc acc cca tgc ctg ggt ccc tgt ccc aag gtg tgc     1065
Ser Asn Leu Leu Cys Thr Pro Cys Leu Gly Pro Cys Pro Lys Val Cys
    325                 330                 335

cac ctc cta gaa ggc gag aag acc atc gac tcg gtg acg tct gcc cag     1113
His Leu Leu Glu Gly Glu Lys Thr Ile Asp Ser Val Thr Ser Ala Gln
340                 345                 350                 355

gag ctc cga gga tgc acc gtc atc aac ggg agt ctg atc atc aac att     1161
Glu Leu Arg Gly Cys Thr Val Ile Asn Gly Ser Leu Ile Ile Asn Ile
                360                 365                 370

cga gga ggc aac aat ctg gca gct gag cta gaa gcc aac ctc ggc ctc     1209
Arg Gly Gly Asn Asn Leu Ala Ala Glu Leu Glu Ala Asn Leu Gly Leu
            375                 380                 385

att gaa gaa att tca ggg tat cta aaa atc cgc cga tcc tac gct ctg     1257
Ile Glu Glu Ile Ser Gly Tyr Leu Lys Ile Arg Arg Ser Tyr Ala Leu
        390                 395                 400

gtg tca ctt tcc ttc ttc cgg aag tta cgt ctg att cga gga gag acc     1305
Val Ser Leu Ser Phe Phe Arg Lys Leu Arg Leu Ile Arg Gly Glu Thr
    405                 410                 415

ttg gaa att ggg aac tac tcc ttc tat gcc ttg gac aac cag aac cta     1353
Leu Glu Ile Gly Asn Tyr Ser Phe Tyr Ala Leu Asp Asn Gln Asn Leu
420                 425                 430                 435

agg cag ctc tgg gac tgg agc aaa cac aac ctc acc atc act cag ggg     1401
Arg Gln Leu Trp Asp Trp Ser Lys His Asn Leu Thr Ile Thr Gln Gly
                440                 445                 450

aaa ctc ttc ttc cac tat aac ccc aaa ctc tgc ttg tca gaa atc cac     1449
Lys Leu Phe Phe His Tyr Asn Pro Lys Leu Cys Leu Ser Glu Ile His
            455                 460                 465

aag atg gaa gaa gtt tca gga acc aag ggg cgc cag gag aga aac gac     1497
Lys Met Glu Glu Val Ser Gly Thr Lys Gly Arg Gln Glu Arg Asn Asp
        470                 475                 480

att gcc ctg aag acc aat ggg gac cag gca tcc tgt gaa aat gag tta     1545
Ile Ala Leu Lys Thr Asn Gly Asp Gln Ala Ser Cys Glu Asn Glu Leu
    485                 490                 495

ctt aaa ttt tct tac att cgg aca tct ttt gac aag atc ttg ctg aga     1593
Leu Lys Phe Ser Tyr Ile Arg Thr Ser Phe Asp Lys Ile Leu Leu Arg
500                 505                 510                 515

tgg gag ccg tac tgg ccc ccc gac ttc cga gac ctc ttg ggg ttc atg     1641
Trp Glu Pro Tyr Trp Pro Pro Asp Phe Arg Asp Leu Leu Gly Phe Met
                520                 525                 530

ctg ttc tac aaa gag gcc cct tat cag aat gtg acg gag ttc gac ggg     1689
Leu Phe Tyr Lys Glu Ala Pro Tyr Gln Asn Val Thr Glu Phe Asp Gly
            535                 540                 545
```

```
cag gat gca tgt ggt tcc aac agt tgg acg gtg gta gac att gac cca      1737
Gln Asp Ala Cys Gly Ser Asn Ser Trp Thr Val Val Asp Ile Asp Pro
        550                 555                 560

ccc ctg agg tcc aac gac ccc aaa tca cag aac cac cca ggg tgg ctg      1785
Pro Leu Arg Ser Asn Asp Pro Lys Ser Gln Asn His Pro Gly Trp Leu
    565                 570                 575

atg cgg ggt ctc aag ccc tgg acc cag tat gcc atc ttt gtg aag acc      1833
Met Arg Gly Leu Lys Pro Trp Thr Gln Tyr Ala Ile Phe Val Lys Thr
580                 585                 590                 595

ctg gtc acc ttt tcg gat gaa cgc cgg acc tat ggg gcc aag agt gac      1881
Leu Val Thr Phe Ser Asp Glu Arg Arg Thr Tyr Gly Ala Lys Ser Asp
                600                 605                 610

atc att tat gtc cag aca gat gcc acc aac ccc tct gtg ccc ctg gat      1929
Ile Ile Tyr Val Gln Thr Asp Ala Thr Asn Pro Ser Val Pro Leu Asp
            615                 620                 625

cca atc tca gtg tct aac tca tca tcc cag att att ctg aag tgg aaa      1977
Pro Ile Ser Val Ser Asn Ser Ser Ser Gln Ile Ile Leu Lys Trp Lys
        630                 635                 640

cca ccc tcc gac ccc aat ggc aac atc acc cac tac ctg gtt ttc tgg      2025
Pro Pro Ser Asp Pro Asn Gly Asn Ile Thr His Tyr Leu Val Phe Trp
    645                 650                 655

gag agg cag gcg gaa gac agt gag ctg ttc gag ctg gat tat tgc ctc      2073
Glu Arg Gln Ala Glu Asp Ser Glu Leu Phe Glu Leu Asp Tyr Cys Leu
660                 665                 670                 675

aaa ggg ctg aag ctg ccc tcg agg acc tgg tct cca cca ttc gag tct      2121
Lys Gly Leu Lys Leu Pro Ser Arg Thr Trp Ser Pro Pro Phe Glu Ser
                680                 685                 690

gaa gat tct cag aag cac aac cag agt gag tat gag gat tcg gcc ggc      2169
Glu Asp Ser Gln Lys His Asn Gln Ser Glu Tyr Glu Asp Ser Ala Gly
            695                 700                 705

gaa tgc tgc tcc tgt cca aag aca gac tct cag atc ctg aag gag ctg      2217
Glu Cys Cys Ser Cys Pro Lys Thr Asp Ser Gln Ile Leu Lys Glu Leu
        710                 715                 720

gag gag tcc tcg ttt agg aag acg ttt gag gat tac ctg cac aac gtg      2265
Glu Glu Ser Ser Phe Arg Lys Thr Phe Glu Asp Tyr Leu His Asn Val
    725                 730                 735

gtt ttc gtc ccc agg cca tct cgg aaa cgc agg tcc ctt ggc gat gtt      2313
Val Phe Val Pro Arg Pro Ser Arg Lys Arg Arg Ser Leu Gly Asp Val
740                 745                 750                 755

ggg aat gtg acg gtg gcc gtg ccc acg gtg gca gct ttc ccc aac act      2361
Gly Asn Val Thr Val Ala Val Pro Thr Val Ala Ala Phe Pro Asn Thr
                760                 765                 770

tcc tcg acc agc gtg ccc acg agt ccg gag gag cac agg cct ttt gag      2409
Ser Ser Thr Ser Val Pro Thr Ser Pro Glu Glu His Arg Pro Phe Glu
            775                 780                 785

aag gtg gtg aac aag gag tcg ctg gtc atc tcc ggc ttg cga cac ttc      2457
Lys Val Val Asn Lys Glu Ser Leu Val Ile Ser Gly Leu Arg His Phe
```

790 795 800

acg ggc tat cgc atc gag ctg cag gct tgc aac cag gac acc cct gag 2505
Thr Gly Tyr Arg Ile Glu Leu Gln Ala Cys Asn Gln Asp Thr Pro Glu
805 810 815

gaa cgg tgc agt gtg gca gcc tac gtc agt gcg agg acc atg cct gaa 2553
Glu Arg Cys Ser Val Ala Ala Tyr Val Ser Ala Arg Thr Met Pro Glu
820 825 830 835

gcc aag gct gat gac att gtt ggc cct gtg acg cat gaa atc ttt gag 2601
Ala Lys Ala Asp Asp Ile Val Gly Pro Val Thr His Glu Ile Phe Glu
840 845 850

aac aac gtc gtc cac ttg atg tgg cag gag ccg aag gag ccc aat ggt 2649
Asn Asn Val Val His Leu Met Trp Gln Glu Pro Lys Glu Pro Asn Gly
855 860 865

ctg atc gtg ctg tat gaa gtg agt tat cgg cga tat ggt gat gag gag 2697
Leu Ile Val Leu Tyr Glu Val Ser Tyr Arg Arg Tyr Gly Asp Glu Glu
870 875 880

ctg cat ctc tgc gtc tcc cgc aag cac ttc gct ctg gaa cgg ggc tgc 2745
Leu His Leu Cys Val Ser Arg Lys His Phe Ala Leu Glu Arg Gly Cys
885 890 895

agg ctg cgt ggg ctg tca ccg ggg aac tac agc gtg cga atc cgg gcc 2793
Arg Leu Arg Gly Leu Ser Pro Gly Asn Tyr Ser Val Arg Ile Arg Ala
900 905 910 915

acc tcc ctt gcg ggc aac ggc tct tgg acg gaa ccc acc tat ttc tac 2841
Thr Ser Leu Ala Gly Asn Gly Ser Trp Thr Glu Pro Thr Tyr Phe Tyr
920 925 930

gtg aca gac tat tta gac gtc ccg tca aat att gca aaa att atc atc 2889
Val Thr Asp Tyr Leu Asp Val Pro Ser Asn Ile Ala Lys Ile Ile Ile
935 940 945

ggc ccc ctc atc ttt gtc ttt ctc ttc agt gtt gtg att gga agt att 2937
Gly Pro Leu Ile Phe Val Phe Leu Phe Ser Val Val Ile Gly Ser Ile
950 955 960

tat cta ttc ctg aga aag agg cag cca gat ggg ccg ctg gga ccg ctt 2985
Tyr Leu Phe Leu Arg Lys Arg Gln Pro Asp Gly Pro Leu Gly Pro Leu
965 970 975

tac gct tct tca aac cct gag tat ctc agt gcc agt gat gtg ttt cca 3033
Tyr Ala Ser Ser Asn Pro Glu Tyr Leu Ser Ala Ser Asp Val Phe Pro
980 985 990 995

tgc tct gtg tac gtg ccg gac gag tgg gag gtg tct cga gag aag 3078
Cys Ser Val Tyr Val Pro Asp Glu Trp Glu Val Ser Arg Glu Lys
1000 1005 1010

atc acc ctc ctt cga gag ctg ggg cag ggc tcc ttc ggc atg gtg 3123
Ile Thr Leu Leu Arg Glu Leu Gly Gln Gly Ser Phe Gly Met Val
1015 1020 1025

tat gag ggc aat gcc agg gac atc atc aag ggt gag gca gag acc 3168
Tyr Glu Gly Asn Ala Arg Asp Ile Ile Lys Gly Glu Ala Glu Thr
1030 1035 1040

cgc gtg gcg gtg aag acg gtc aac gag tca gcc agt ctc cga gag 3213

```
Arg Val Ala Val Lys  Thr Val Asn Glu Ser  Ala Ser Leu Arg Glu
                1045                1050                1055

cgg att gag ttc ctc  aat gag gcc tcg gtc  atg aag ggc ttc acc      3258
Arg Ile Glu Phe Leu  Asn Glu Ala Ser Val  Met Lys Gly Phe Thr
                1060                1065                1070

tgc cat cac gtg gtg  cgc ctc ctg gga gtg  gtg tcc aag ggc cag      3303
Cys His His Val Val  Arg Leu Leu Gly Val  Val Ser Lys Gly Gln
                1075                1080                1085

ccc acg ctg gtg gtg  atg gag ctg atg gct  cac gga gac ctg aag      3348
Pro Thr Leu Val Val  Met Glu Leu Met Ala  His Gly Asp Leu Lys
                1090                1095                1100

agc tac ctc cgt tct  ctg cgg cca gag gct  gag aat aat cct ggc      3393
Ser Tyr Leu Arg Ser  Leu Arg Pro Glu Ala  Glu Asn Asn Pro Gly
                1105                1110                1115

cgc cct ccc cct acc  ctt caa gag atg att  cag atg gcg gca gag      3438
Arg Pro Pro Pro Thr  Leu Gln Glu Met Ile  Gln Met Ala Ala Glu
                1120                1125                1130

att gct gac ggg atg  gcc tac ctg aac gcc  aag aag ttt gtg cat      3483
Ile Ala Asp Gly Met  Ala Tyr Leu Asn Ala  Lys Lys Phe Val His
                1135                1140                1145

cgg gac ctg gca gcg  aga aac tgc atg gtc  gcc cat gat ttt act      3528
Arg Asp Leu Ala Ala  Arg Asn Cys Met Val  Ala His Asp Phe Thr
                1150                1155                1160

gtc aaa att gga gac  ttt gga atg acc aga  gac atc tat gaa acg      3573
Val Lys Ile Gly Asp  Phe Gly Met Thr Arg  Asp Ile Tyr Glu Thr
                1165                1170                1175

gat tac tac cgg aaa  ggg ggc aag ggt ctg  ctc cct gta cgg tgg      3618
Asp Tyr Tyr Arg Lys  Gly Gly Lys Gly Leu  Leu Pro Val Arg Trp
                1180                1185                1190

atg gca ccg gag tcc  ctg aag gat ggg gtc  ttc acc act tct tct      3663
Met Ala Pro Glu Ser  Leu Lys Asp Gly Val  Phe Thr Thr Ser Ser
                1195                1200                1205

gac atg tgg tcc ttt  ggc gtg gtc ctt tgg  gaa atc acc agc ttg      3708
Asp Met Trp Ser Phe  Gly Val Val Leu Trp  Glu Ile Thr Ser Leu
                1210                1215                1220

gca gaa cag cct tac  caa ggc ctg tct aat  gaa cag gtg ttg aaa      3753
Ala Glu Gln Pro Tyr  Gln Gly Leu Ser Asn  Glu Gln Val Leu Lys
                1225                1230                1235

ttt gtc atg gat gga  ggg tat ctg gat caa  ccc gac aac tgt cca      3798
Phe Val Met Asp Gly  Gly Tyr Leu Asp Gln  Pro Asp Asn Cys Pro
                1240                1245                1250

gag aga gtc act gac  ctc atg cgc atg tgc  tgg caa ttc aac ccc      3843
Glu Arg Val Thr Asp  Leu Met Arg Met Cys  Trp Gln Phe Asn Pro
                1255                1260                1265

aac atg agg cca acc  ttc ctg gag att gtc  aac ctg ctc aag gac      3888
Asn Met Arg Pro Thr  Phe Leu Glu Ile Val  Asn Leu Leu Lys Asp
                1270                1275                1280
```

gac ctg cac ccc agc ttt cca gag gtg tcg ttc ttc cac agc gag 3933
Asp Leu His Pro Ser Phe Pro Glu Val Ser Phe Phe His Ser Glu
1285 1290 1295

gag aac aag gct ccc gag agt gag gag ctg gag atg gag ttt gag 3978
Glu Asn Lys Ala Pro Glu Ser Glu Glu Leu Glu Met Glu Phe Glu
1300 1305 1310

gac atg gag aat gtg ccc ctg gac cgt tcc tcg cac tgt cag agg 4023
Asp Met Glu Asn Val Pro Leu Asp Arg Ser Ser His Cys Gln Arg
1315 1320 1325

gag gag gcg ggg ggc cgg gat gga ggg tcc tcg ctg ggt ttc aag 4068
Glu Glu Ala Gly Gly Arg Asp Gly Gly Ser Ser Leu Gly Phe Lys
1330 1335 1340

cgg agc tac gag gaa cac atc cct tac aca cac atg aac gga ggc 4113
Arg Ser Tyr Glu Glu His Ile Pro Tyr Thr His Met Asn Gly Gly
1345 1350 1355

aag aaa aac ggg cgg att ctg acc ttg cct cgg tcc aat cct tcc 4158
Lys Lys Asn Gly Arg Ile Leu Thr Leu Pro Arg Ser Asn Pro Ser
1360 1365 1370

taa cagtgcctac cgtggcgggg gcgggcaggg gttcccattt tcgctttcct 4211

ctggtttgaa agcctctgga aaactcagga ttctcacgac tctaccatgt ccaatggagt 4271

tcagagatcg ttcctataca tttctgttca tcttaaggtg gactcgtttg gttaccaatt 4331

taactagtcc tgcagaggat ttaactgtga acctggaggg caaggggttt ccacagttgc 4391

tgctcctttg gggcaacgac ggtttcaaac caggattttg tgtttttcg ttccccccac 4451

ccgcccccag cagatggaaa gaaagcacct gtttttacaa attctttttt ttttttttt 4511

tttttgctgg tgtctgagct tcagtataaa agacaaaact tcctgtttgt ggaacaaaag 4571

ttcgaaagaa aaaacaaaac aaaaacaccc agccctgttc caggagaatt tcaagtttta 4631

caggttgagc ttcaagatgg ttttttggt ttttttttt tctctcatcc aggctgaagg 4691

attttttttt tctttacaaa atgagttcct caaattgacc aatagctgct gctttcatat 4751

tttggataag ggtctgtggt cccggcgtgt gctcacgtgt gtatgcacgt gtgtgtgtcc 4811

attagacacg gctgacgtgt gtgcaaagta tccatgcgga gttgatgctt tgggaattgg 4871

ctcatgaagg ttcttctcaa gggtgcgagc tcatcccccc ctctccttcc ttcttattga 4931

ctgggagact gtgctctcga cagattcttc ttgtgtcaga agtctagcct caggtttcta 4991

ccctcccttc acattggtgg ccaagggagg agcatttcat ttggagtgat tatgaatctt 5051

ttcaagacca aaccaagcta ggacattaaa aaaaaaaaaa agaaaaagaa agaaaaaaca 5111

aaatggaaaa aggaaaaaaa aaaagaactg agatgacaga gttttgagaa tatatttgta 5171

ccatattta 5180

<210> 12
<211> 1370

<212> PRT
<213> Homo sapiens

<400> 12

Met Gly Thr Gly Gly Arg Arg Gly Ala Ala Ala Pro Leu Leu Val
1 5 10 15

Ala Val Ala Ala Leu Leu Leu Gly Ala Ala Gly His Leu Tyr Pro Gly
20 25 30

Glu Val Cys Pro Gly Met Asp Ile Arg Asn Asn Leu Thr Arg Leu His
35 40 45

Glu Leu Glu Asn Cys Ser Val Ile Glu Gly His Leu Gln Ile Leu Leu
50 55 60

Met Phe Lys Thr Arg Pro Glu Asp Phe Arg Asp Leu Ser Phe Pro Lys
65 70 75 80

Leu Ile Met Ile Thr Asp Tyr Leu Leu Leu Phe Arg Val Tyr Gly Leu
85 90 95

Glu Ser Leu Lys Asp Leu Phe Pro Asn Leu Thr Val Ile Arg Gly Ser
100 105 110

Arg Leu Phe Phe Asn Tyr Ala Leu Val Ile Phe Glu Met Val His Leu
115 120 125

Lys Glu Leu Gly Leu Tyr Asn Leu Met Asn Ile Thr Arg Gly Ser Val
130 135 140

Arg Ile Glu Lys Asn Asn Glu Leu Cys Tyr Leu Ala Thr Ile Asp Trp
145 150 155 160

Ser Arg Ile Leu Asp Ser Val Glu Asp Asn Tyr Ile Val Leu Asn Lys
165 170 175

Asp Asp Asn Glu Glu Cys Gly Asp Ile Cys Pro Gly Thr Ala Lys Gly
180 185 190

Lys Thr Asn Cys Pro Ala Thr Val Ile Asn Gly Gln Phe Val Glu Arg
195 200 205

Cys Trp Thr His Ser His Cys Gln Lys Val Cys Pro Thr Ile Cys Lys
210 215 220

Ser His Gly Cys Thr Ala Glu Gly Leu Cys Cys His Ser Glu Cys Leu
225 230 235 240

Gly Asn Cys Ser Gln Pro Asp Asp Pro Thr Lys Cys Val Ala Cys Arg
245 250 255

Asn Phe Tyr Leu Asp Gly Arg Cys Val Glu Thr Cys Pro Pro Pro Tyr
260 265 270

Tyr His Phe Gln Asp Trp Arg Cys Val Asn Phe Ser Phe Cys Gln Asp
275 280 285

Leu His His Lys Cys Lys Asn Ser Arg Arg Gln Gly Cys His Gln Tyr
290 295 300

Val Ile His Asn Asn Lys Cys Ile Pro Glu Cys Pro Ser Gly Tyr Thr
305 310 315 320

Met Asn Ser Ser Asn Leu Leu Cys Thr Pro Cys Leu Gly Pro Cys Pro
325 330 335

Lys Val Cys His Leu Leu Glu Gly Glu Lys Thr Ile Asp Ser Val Thr
340 345 350

Ser Ala Gln Glu Leu Arg Gly Cys Thr Val Ile Asn Gly Ser Leu Ile
355 360 365

Ile Asn Ile Arg Gly Gly Asn Asn Leu Ala Ala Glu Leu Glu Ala Asn
370 375 380

Leu Gly Leu Ile Glu Glu Ile Ser Gly Tyr Leu Lys Ile Arg Arg Ser
385 390 395 400

Tyr Ala Leu Val Ser Leu Ser Phe Phe Arg Lys Leu Arg Leu Ile Arg
405 410 415

Gly Glu Thr Leu Glu Ile Gly Asn Tyr Ser Phe Tyr Ala Leu Asp Asn
420 425 430

Gln Asn Leu Arg Gln Leu Trp Asp Trp Ser Lys His Asn Leu Thr Ile
435 440 445

Thr Gln Gly Lys Leu Phe Phe His Tyr Asn Pro Lys Leu Cys Leu Ser
450 455 460

Glu Ile His Lys Met Glu Glu Val Ser Gly Thr Lys Gly Arg Gln Glu
465 470 475 480

Arg Asn Asp Ile Ala Leu Lys Thr Asn Gly Asp Gln Ala Ser Cys Glu

485 490 495

Asn Glu Leu Leu Lys Phe Ser Tyr Ile Arg Thr Ser Phe Asp Lys Ile
500 505 510

Leu Leu Arg Trp Glu Pro Tyr Trp Pro Pro Asp Phe Arg Asp Leu Leu
515 520 525

Gly Phe Met Leu Phe Tyr Lys Glu Ala Pro Tyr Gln Asn Val Thr Glu
530 535 540

Phe Asp Gly Gln Asp Ala Cys Gly Ser Asn Ser Trp Thr Val Val Asp
545 550 555 560

Ile Asp Pro Pro Leu Arg Ser Asn Asp Pro Lys Ser Gln Asn His Pro
565 570 575

Gly Trp Leu Met Arg Gly Leu Lys Pro Trp Thr Gln Tyr Ala Ile Phe
580 585 590

Val Lys Thr Leu Val Thr Phe Ser Asp Glu Arg Arg Thr Tyr Gly Ala
595 600 605

Lys Ser Asp Ile Ile Tyr Val Gln Thr Asp Ala Thr Asn Pro Ser Val
610 615 620

Pro Leu Asp Pro Ile Ser Val Ser Asn Ser Ser Ser Gln Ile Ile Leu
625 630 635 640

Lys Trp Lys Pro Pro Ser Asp Pro Asn Gly Asn Ile Thr His Tyr Leu
645 650 655

Val Phe Trp Glu Arg Gln Ala Glu Asp Ser Glu Leu Phe Glu Leu Asp
660 665 670

Tyr Cys Leu Lys Gly Leu Lys Leu Pro Ser Arg Thr Trp Ser Pro Pro
675 680 685

Phe Glu Ser Glu Asp Ser Gln Lys His Asn Gln Ser Glu Tyr Glu Asp
690 695 700

Ser Ala Gly Glu Cys Cys Ser Cys Pro Lys Thr Asp Ser Gln Ile Leu
705 710 715 720

Lys Glu Leu Glu Glu Ser Ser Phe Arg Lys Thr Phe Glu Asp Tyr Leu
725 730 735

His Asn Val Val Phe Val Pro Arg Pro Ser Arg Lys Arg Arg Ser Leu
740 745 750

Gly Asp Val Gly Asn Val Thr Val Ala Val Pro Thr Val Ala Ala Phe
755 760 765

Pro Asn Thr Ser Ser Thr Ser Val Pro Thr Ser Pro Glu Glu His Arg
770 775 780

Pro Phe Glu Lys Val Val Asn Lys Glu Ser Leu Val Ile Ser Gly Leu
785 790 795 800

Arg His Phe Thr Gly Tyr Arg Ile Glu Leu Gln Ala Cys Asn Gln Asp
805 810 815

Thr Pro Glu Glu Arg Cys Ser Val Ala Ala Tyr Val Ser Ala Arg Thr
820 825 830

Met Pro Glu Ala Lys Ala Asp Asp Ile Val Gly Pro Val Thr His Glu
835 840 845

Ile Phe Glu Asn Asn Val Val His Leu Met Trp Gln Glu Pro Lys Glu
850 855 860

Pro Asn Gly Leu Ile Val Leu Tyr Glu Val Ser Tyr Arg Arg Tyr Gly
865 870 875 880

Asp Glu Glu Leu His Leu Cys Val Ser Arg Lys His Phe Ala Leu Glu
885 890 895

Arg Gly Cys Arg Leu Arg Gly Leu Ser Pro Gly Asn Tyr Ser Val Arg
900 905 910

Ile Arg Ala Thr Ser Leu Ala Gly Asn Gly Ser Trp Thr Glu Pro Thr
915 920 925

Tyr Phe Tyr Val Thr Asp Tyr Leu Asp Val Pro Ser Asn Ile Ala Lys
930 935 940

Ile Ile Ile Gly Pro Leu Ile Phe Val Phe Leu Phe Ser Val Val Ile
945 950 955 960

Gly Ser Ile Tyr Leu Phe Leu Arg Lys Arg Gln Pro Asp Gly Pro Leu
965 970 975

Gly Pro Leu Tyr Ala Ser Ser Asn Pro Glu Tyr Leu Ser Ala Ser Asp
980 985 990

Val Phe Pro Cys Ser Val Tyr Val Pro Asp Glu Trp Glu Val Ser Arg
995 1000 1005

Glu Lys Ile Thr Leu Leu Arg Glu Leu Gly Gln Gly Ser Phe Gly
1010 1015 1020

Met Val Tyr Glu Gly Asn Ala Arg Asp Ile Ile Lys Gly Glu Ala
1025 1030 1035

Glu Thr Arg Val Ala Val Lys Thr Val Asn Glu Ser Ala Ser Leu
1040 1045 1050

Arg Glu Arg Ile Glu Phe Leu Asn Glu Ala Ser Val Met Lys Gly
1055 1060 1065

Phe Thr Cys His His Val Val Arg Leu Leu Gly Val Val Ser Lys
1070 1075 1080

Gly Gln Pro Thr Leu Val Val Met Glu Leu Met Ala His Gly Asp
1085 1090 1095

Leu Lys Ser Tyr Leu Arg Ser Leu Arg Pro Glu Ala Glu Asn Asn
1100 1105 1110

Pro Gly Arg Pro Pro Pro Thr Leu Gln Glu Met Ile Gln Met Ala
1115 1120 1125

Ala Glu Ile Ala Asp Gly Met Ala Tyr Leu Asn Ala Lys Lys Phe
1130 1135 1140

Val His Arg Asp Leu Ala Ala Arg Asn Cys Met Val Ala His Asp
1145 1150 1155

Phe Thr Val Lys Ile Gly Asp Phe Gly Met Thr Arg Asp Ile Tyr
1160 1165 1170

Glu Thr Asp Tyr Tyr Arg Lys Gly Gly Lys Gly Leu Leu Pro Val
1175 1180 1185

Arg Trp Met Ala Pro Glu Ser Leu Lys Asp Gly Val Phe Thr Thr
1190 1195 1200

Ser Ser Asp Met Trp Ser Phe Gly Val Val Leu Trp Glu Ile Thr
1205 1210 1215

Ser Leu Ala Glu Gln Pro Tyr Gln Gly Leu Ser Asn Glu Gln Val
1220 1225 1230

Leu Lys Phe Val Met Asp Gly Gly Tyr Leu Asp Gln Pro Asp Asn
1235 1240 1245

Cys Pro Glu Arg Val Thr Asp Leu Met Arg Met Cys Trp Gln Phe
1250 1255 1260

Asn Pro Asn Met Arg Pro Thr Phe Leu Glu Ile Val Asn Leu Leu
1265 1270 1275

Lys Asp Asp Leu His Pro Ser Phe Pro Glu Val Ser Phe Phe His
1280 1285 1290

Ser Glu Glu Asn Lys Ala Pro Glu Ser Glu Glu Leu Glu Met Glu
1295 1300 1305

Phe Glu Asp Met Glu Asn Val Pro Leu Asp Arg Ser Ser His Cys
1310 1315 1320

Gln Arg Glu Glu Ala Gly Gly Arg Asp Gly Gly Ser Ser Leu Gly
1325 1330 1335

Phe Lys Arg Ser Tyr Glu Glu His Ile Pro Tyr Thr His Met Asn
1340 1345 1350

Gly Gly Lys Lys Asn Gly Arg Ile Leu Thr Leu Pro Arg Ser Asn
1355 1360 1365

Pro Ser
1370

<210> 13
<211> 5084
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (22)..(2952)

<220>
<221> variation
<222> (3101)..(3101)
<223> C and T alleles exist at this position

<220>
<221> variation
<222> (4354)..(4354)
<223> A and G alleles exist at this position

<220>

<221> variation
<222> (4656)..(4656)
<223> G and T alleles exist at this position

<400> 13

```
gatcccatcg cagctaccgc g atg aga ggc gct cgc ggc gcc tgg gat ttt        51
                        Met Arg Gly Ala Arg Gly Ala Trp Asp Phe
                        1               5                   10

ctc tgc gtt ctg ctc cta ctg ctt cgc gtc cag aca ggc tct tct caa        99
Leu Cys Val Leu Leu Leu Leu Leu Arg Val Gln Thr Gly Ser Ser Gln
                15                  20                  25

cca tct gtg agt cca ggg gaa ccg tct cca cca tcc atc cat cca gga       147
Pro Ser Val Ser Pro Gly Glu Pro Ser Pro Pro Ser Ile His Pro Gly
            30                  35                  40

aaa tca gac tta ata gtc cgc gtg ggc gac gag att agg ctg tta tgc       195
Lys Ser Asp Leu Ile Val Arg Val Gly Asp Glu Ile Arg Leu Leu Cys
        45                  50                  55

act gat ccg ggc ttt gtc aaa tgg act ttt gag atc ctg gat gaa acg       243
Thr Asp Pro Gly Phe Val Lys Trp Thr Phe Glu Ile Leu Asp Glu Thr
    60                  65                  70

aat gag aat aag cag aat gaa tgg atc acg gaa aag gca gaa gcc acc       291
Asn Glu Asn Lys Gln Asn Glu Trp Ile Thr Glu Lys Ala Glu Ala Thr
75                  80                  85                  90

aac acc ggc aaa tac acg tgc acc aac aaa cac ggc tta agc aat tcc       339
Asn Thr Gly Lys Tyr Thr Cys Thr Asn Lys His Gly Leu Ser Asn Ser
                95                  100                 105

att tat gtg ttt gtt aga gat cct gcc aag ctt ttc ctt gtt gac cgc       387
Ile Tyr Val Phe Val Arg Asp Pro Ala Lys Leu Phe Leu Val Asp Arg
            110                 115                 120

tcc ttg tat ggg aaa gaa gac aac gac acg ctg gtc cgc tgt cct ctc       435
Ser Leu Tyr Gly Lys Glu Asp Asn Asp Thr Leu Val Arg Cys Pro Leu
        125                 130                 135

aca gac cca gaa gtg acc aat tat tcc ctc aag ggg tgc cag ggg aag       483
Thr Asp Pro Glu Val Thr Asn Tyr Ser Leu Lys Gly Cys Gln Gly Lys
    140                 145                 150

cct ctt ccc aag gac ttg agg ttt att cct gac ccc aag gcg ggc atc       531
Pro Leu Pro Lys Asp Leu Arg Phe Ile Pro Asp Pro Lys Ala Gly Ile
155                 160                 165                 170

atg atc aaa agt gtg aaa cgc gcc tac cat cgg ctc tgt ctg cat tgt       579
Met Ile Lys Ser Val Lys Arg Ala Tyr His Arg Leu Cys Leu His Cys
                175                 180                 185

tct gtg gac cag gag ggc aag tca gtg ctg tcg gaa aaa ttc atc ctg       627
Ser Val Asp Gln Glu Gly Lys Ser Val Leu Ser Glu Lys Phe Ile Leu
            190                 195                 200

aaa gtg agg cca gcc ttc aaa gct gtg cct gtt gtg tct gtg tcc aaa       675
Lys Val Arg Pro Ala Phe Lys Ala Val Pro Val Val Ser Val Ser Lys
        205                 210                 215

gca agc tat ctt ctt agg gaa ggg gaa gaa ttc aca gtg acg tgc aca       723
Ala Ser Tyr Leu Leu Arg Glu Gly Glu Glu Phe Thr Val Thr Cys Thr
```

220 225 230

ata aaa gat gtg tct agt tct gtg tac tca acg tgg aaa aga gaa aac 771
Ile Lys Asp Val Ser Ser Ser Val Tyr Ser Thr Trp Lys Arg Glu Asn
235 240 245 250

agt cag act aaa cta cag gag aaa tat aat agc tgg cat cac ggt gac 819
Ser Gln Thr Lys Leu Gln Glu Lys Tyr Asn Ser Trp His His Gly Asp
255 260 265

ttc aat tat gaa cgt cag gca acg ttg act atc agt tca gcg aga gtt 867
Phe Asn Tyr Glu Arg Gln Ala Thr Leu Thr Ile Ser Ser Ala Arg Val
270 275 280

aat gat tct gga gtg ttc atg tgt tat gcc aat aat act ttt gga tca 915
Asn Asp Ser Gly Val Phe Met Cys Tyr Ala Asn Asn Thr Phe Gly Ser
285 290 295

gca aat gtc aca aca acc ttg gaa gta gta gat aaa gga ttc att aat 963
Ala Asn Val Thr Thr Thr Leu Glu Val Val Asp Lys Gly Phe Ile Asn
300 305 310

atc ttc ccc atg ata aac act aca gta ttt gta aac gat gga gaa aat 1011
Ile Phe Pro Met Ile Asn Thr Thr Val Phe Val Asn Asp Gly Glu Asn
315 320 325 330

gta gat ttg att gtt gaa tat gaa gca ttc ccc aaa cct gaa cac cag 1059
Val Asp Leu Ile Val Glu Tyr Glu Ala Phe Pro Lys Pro Glu His Gln
335 340 345

cag tgg atc tat atg aac aga acc ttc act gat aaa tgg gaa gat tat 1107
Gln Trp Ile Tyr Met Asn Arg Thr Phe Thr Asp Lys Trp Glu Asp Tyr
350 355 360

ccc aag tct gag aat gaa agt aat atc aga tac gta agt gaa ctt cat 1155
Pro Lys Ser Glu Asn Glu Ser Asn Ile Arg Tyr Val Ser Glu Leu His
365 370 375

cta acg aga tta aaa ggc acc gaa gga ggc act tac aca ttc cta gtg 1203
Leu Thr Arg Leu Lys Gly Thr Glu Gly Gly Thr Tyr Thr Phe Leu Val
380 385 390

tcc aat tct gac gtc aat gct gcc ata gca ttt aat gtt tat gtg aat 1251
Ser Asn Ser Asp Val Asn Ala Ala Ile Ala Phe Asn Val Tyr Val Asn
395 400 405 410

aca aaa cca gaa atc ctg act tac gac agg ctc gtg aat ggc atg ctc 1299
Thr Lys Pro Glu Ile Leu Thr Tyr Asp Arg Leu Val Asn Gly Met Leu
415 420 425

caa tgt gtg gca gca gga ttc cca gag ccc aca ata gat tgg tat ttt 1347
Gln Cys Val Ala Ala Gly Phe Pro Glu Pro Thr Ile Asp Trp Tyr Phe
430 435 440

tgt cca gga act gag cag aga tgc tct gct tct gta ctg cca gtg gat 1395
Cys Pro Gly Thr Glu Gln Arg Cys Ser Ala Ser Val Leu Pro Val Asp
445 450 455

gtg cag aca cta aac tca tct ggg cca ccg ttt gga aag cta gtg gtt 1443
Val Gln Thr Leu Asn Ser Ser Gly Pro Pro Phe Gly Lys Leu Val Val
460 465 470

cag agt tct ata gat tct agt gca ttc aag cac aat ggc acg gtt gaa 1491

```
Gln Ser Ser Ile Asp Ser Ser Ala Phe Lys His Asn Gly Thr Val Glu
475                 480                 485                 490

tgt aag gct tac aac gat gtg ggc aag act tct gcc tat ttt aac ttt      1539
Cys Lys Ala Tyr Asn Asp Val Gly Lys Thr Ser Ala Tyr Phe Asn Phe
                495                 500                 505

gca ttt aaa ggt aac aac aaa gag caa atc cat ccc cac acc ctg ttc      1587
Ala Phe Lys Gly Asn Asn Lys Glu Gln Ile His Pro His Thr Leu Phe
            510                 515                 520

act cct ttg ctg att ggt ttc gta atc gta gct ggc atg atg tgc att      1635
Thr Pro Leu Leu Ile Gly Phe Val Ile Val Ala Gly Met Met Cys Ile
        525                 530                 535

att gtg atg att ctg acc tac aaa tat tta cag aaa ccc atg tat gaa      1683
Ile Val Met Ile Leu Thr Tyr Lys Tyr Leu Gln Lys Pro Met Tyr Glu
    540                 545                 550

gta cag tgg aag gtt gtt gag gag ata aat gga aac aat tat gtt tac      1731
Val Gln Trp Lys Val Val Glu Glu Ile Asn Gly Asn Asn Tyr Val Tyr
555                 560                 565                 570

ata gac cca aca caa ctt cct tat gat cac aaa tgg gag ttt ccc aga      1779
Ile Asp Pro Thr Gln Leu Pro Tyr Asp His Lys Trp Glu Phe Pro Arg
                575                 580                 585

aac agg ctg agt ttt ggg aaa acc ctg ggt gct gga gct ttc ggg aag      1827
Asn Arg Leu Ser Phe Gly Lys Thr Leu Gly Ala Gly Ala Phe Gly Lys
            590                 595                 600

gtt gtt gag gca act gct tat ggc tta att aag tca gat gcg gcc atg      1875
Val Val Glu Ala Thr Ala Tyr Gly Leu Ile Lys Ser Asp Ala Ala Met
        605                 610                 615

act gtc gct gta aag atg ctc aag ccg agt gcc cat ttg aca gaa cgg      1923
Thr Val Ala Val Lys Met Leu Lys Pro Ser Ala His Leu Thr Glu Arg
    620                 625                 630

gaa gcc ctc atg tct gaa ctc aaa gtc ctg agt tac ctt ggt aat cac      1971
Glu Ala Leu Met Ser Glu Leu Lys Val Leu Ser Tyr Leu Gly Asn His
635                 640                 645                 650

atg aat att gtg aat cta ctt gga gcc tgc acc att gga ggg ccc acc      2019
Met Asn Ile Val Asn Leu Leu Gly Ala Cys Thr Ile Gly Gly Pro Thr
                655                 660                 665

ctg gtc att aca gaa tat tgt tgc tat ggt gat ctt ttg aat ttt ttg      2067
Leu Val Ile Thr Glu Tyr Cys Cys Tyr Gly Asp Leu Leu Asn Phe Leu
            670                 675                 680

aga aga aaa cgt gat tca ttt att tgt tca aag cag gaa gat cat gca      2115
Arg Arg Lys Arg Asp Ser Phe Ile Cys Ser Lys Gln Glu Asp His Ala
        685                 690                 695

gaa gct gca ctt tat aag aat ctt ctg cat tca aag gag tct tcc tgc      2163
Glu Ala Ala Leu Tyr Lys Asn Leu Leu His Ser Lys Glu Ser Ser Cys
    700                 705                 710

agc gat agt act aat gag tac atg gac atg aaa cct gga gtt tct tat      2211
Ser Asp Ser Thr Asn Glu Tyr Met Asp Met Lys Pro Gly Val Ser Tyr
715                 720                 725                 730
```

```
gtt gtc cca acc aag gcc gac aaa agg aga tct gtg aga ata ggc tca      2259
Val Val Pro Thr Lys Ala Asp Lys Arg Arg Ser Val Arg Ile Gly Ser
                735                 740                 745

tac ata gaa aga gat gtg act ccc gcc atc atg gag gat gac gag ttg      2307
Tyr Ile Glu Arg Asp Val Thr Pro Ala Ile Met Glu Asp Asp Glu Leu
            750                 755                 760

gcc cta gac tta gaa gac ttg ctg agc ttt tct tac cag gtg gca aag      2355
Ala Leu Asp Leu Glu Asp Leu Leu Ser Phe Ser Tyr Gln Val Ala Lys
        765                 770                 775

ggc atg gct ttc ctc gcc tcc aag aat tgt att cac aga gac ttg gca      2403
Gly Met Ala Phe Leu Ala Ser Lys Asn Cys Ile His Arg Asp Leu Ala
    780                 785                 790

gcc aga aat atc ctc ctt act cat ggt cgg atc aca aag att tgt gat      2451
Ala Arg Asn Ile Leu Leu Thr His Gly Arg Ile Thr Lys Ile Cys Asp
795                 800                 805                 810

ttt ggt cta gcc aga gac atc aag aat gat tct aat tat gtg gtt aaa      2499
Phe Gly Leu Ala Arg Asp Ile Lys Asn Asp Ser Asn Tyr Val Val Lys
                815                 820                 825

gga aac gct cga cta cct gtg aag tgg atg gca cct gaa agc att ttc      2547
Gly Asn Ala Arg Leu Pro Val Lys Trp Met Ala Pro Glu Ser Ile Phe
            830                 835                 840

aac tgt gta tac acg ttt gaa agt gac gtc tgg tcc tat ggg att ttt      2595
Asn Cys Val Tyr Thr Phe Glu Ser Asp Val Trp Ser Tyr Gly Ile Phe
        845                 850                 855

ctt tgg gag ctg ttc tct tta gga agc agc ccc tat cct gga atg ccg      2643
Leu Trp Glu Leu Phe Ser Leu Gly Ser Ser Pro Tyr Pro Gly Met Pro
    860                 865                 870

gtc gat tct aag ttc tac aag atg atc aag gaa ggc ttc cgg atg ctc      2691
Val Asp Ser Lys Phe Tyr Lys Met Ile Lys Glu Gly Phe Arg Met Leu
875                 880                 885                 890

agc cct gaa cac gca cct gct gaa atg tat gac ata atg aag act tgc      2739
Ser Pro Glu His Ala Pro Ala Glu Met Tyr Asp Ile Met Lys Thr Cys
                895                 900                 905

tgg gat gca gat ccc cta aaa aga cca aca ttc aag caa att gtt cag      2787
Trp Asp Ala Asp Pro Leu Lys Arg Pro Thr Phe Lys Gln Ile Val Gln
            910                 915                 920

cta att gag aag cag att tca gag agc acc aat cat att tac tcc aac      2835
Leu Ile Glu Lys Gln Ile Ser Glu Ser Thr Asn His Ile Tyr Ser Asn
        925                 930                 935

tta gca aac tgc agc ccc aac cga cag aag ccc gtg gta gac cat tct      2883
Leu Ala Asn Cys Ser Pro Asn Arg Gln Lys Pro Val Val Asp His Ser
    940                 945                 950

gtg cgg atc aat tct gtc ggc agc acc gct tcc tcc tcc cag cct ctg      2931
Val Arg Ile Asn Ser Val Gly Ser Thr Ala Ser Ser Ser Gln Pro Leu
955                 960                 965                 970

ctt gtg cac gac gat gtc tga gcagaatcag tgtttgggtc acccctccag        2982
Leu Val His Asp Asp Val
                975
```

gaatgatctc ttcttttggc ttccatgatg gttatttttct tttctttcaa cttgcatcca 3042

actccaggat agtgggcacc ccactgcaat cctgtctttc tgagcacact ttagtggcyg 3102

atgatttttg tcatcagcca ccatcctatt gcaaaggttc caactgtata tattcccaat 3162

agcaacgtag cttctaccat gaacagaaaa cattctgatt tggaaaaaga gagggaggta 3222

tggactgggg gccagagtcc tttccaaggc ttctccaatt ctgcccaaaa atatggttga 3282

tagtttacct gaataaatgg tagtaatcac agttggcctt cagaaccatc catagtagta 3342

tgatgataca agattagaag ctgaaaacct aagtccttta tgtggaaaac agaacatcat 3402

tagaacaaag gacagagtat gaacacctgg gcttaagaaa tctagtattt catgctggga 3462

atgagacata ggccatgaaa aaaatgatcc ccaagtgtga acaaaagatg ctcttctgtg 3522

gaccactgca tgagctttta tactaccgac ctggttttta aatagagttt gctattagag 3582

cattgaattg gagagaaggc ctccctagcc agcacttgta tatacgcatc tataaattgt 3642

ccgtgttcat acatttgagg ggaaaacacc ataaggtttc gtttctgtat acaaccctgg 3702

cattatgtcc actgtgtata gaagtagatt aagagccata taagtttgaa ggaaacagtt 3762

aataccattt tttaaggaaa caatataacc acaaagcaca gtttgaacaa aatctcctct 3822

tttagctgat gaacttattc tgtagattct gtggaacaag cctatcagct tcagaatggc 3882

attgtactca atggatttga tgctgtttga caaagttact gattcactgc atggctccca 3942

caggagtggg aaaacactgc catcttagtt tggattctta tgtagcagga aataaagtat 4002

aggtttagcc tccttcgcag gcatgtcctg gacaccgggc cagtatctat atatgtgtat 4062

gtacgtttgt atgtgtgtag acaaatattt ggaggggtat ttttgccctg agtccaagag 4122

ggtcctttag tacctgaaaa gtaacttggc tttcattatt agtactgctc ttgtttcttt 4182

tcacatagct gtctagagta gcttaccaga agcttccata gtggtgcaga ggaagtggaa 4242

ggcatcagtc cctatgtatt tgcagttcac ctgcacttaa ggcactctgt tatttagact 4302

catcttactg tacctgttcc ttagaccttc cataatgcta ctgtctcact graacattta 4362

aattttaccc tttagactgt agcctggata ttattcttgt agtttacctc tttaaaaaca 4422

aaacaaaaca aaacaaaaaa ctccccttcc tcactgccca atataaaagg caaatgtgta 4482

catggcagag tttgtgtgtt gtcttgaaag attcaggtat gttgccttta tggtttcccc 4542

cttctacatt tcttagacta catttagaga actgtggccg ttatctggaa gtaaccattt 4602

gcactggagt tctatgctct cgcacctttc caaagttaac agattttggg gttktgttgt 4662

cacccaagag attgttgttt gccatacttt gtctgaaaaa ttcctttgtg tttctattga 4722

cttcaatgat agtaagaaaa gtggttgtta gttatagatg tctaggtact tcaggggcac 4782

ttcattgaga gttttgtctt gccatacttt gtctgaaaaa ttcctttgtg tttctattga 4842

cttcaatgat agtaagaaaa gtggttgtta gttatagatg tctaggtact tcaggggcac 4902

ttcattgaga gttttgtcaa tgtcttttga atattcccaa gcccatgagt ccttgaaaat 4962

atttttttata tatacagtaa ctttatgtgt aaatacataa gcggcgtaag tttaaaggat 5022

gttggtgttc cacgtgtttt attcctgtat gttgtccaat tgttgacagt tctgaagaat 5082

tc 5084

<210> 14
<211> 976
<212> PRT
<213> Homo sapiens

<400> 14

Met Arg Gly Ala Arg Gly Ala Trp Asp Phe Leu Cys Val Leu Leu Leu
1 5 10 15

Leu Leu Arg Val Gln Thr Gly Ser Ser Gln Pro Ser Val Ser Pro Gly
20 25 30

Glu Pro Ser Pro Pro Ser Ile His Pro Gly Lys Ser Asp Leu Ile Val
35 40 45

Arg Val Gly Asp Glu Ile Arg Leu Leu Cys Thr Asp Pro Gly Phe Val
50 55 60

Lys Trp Thr Phe Glu Ile Leu Asp Glu Thr Asn Glu Asn Lys Gln Asn
65 70 75 80

Glu Trp Ile Thr Glu Lys Ala Glu Ala Thr Asn Thr Gly Lys Tyr Thr
85 90 95

Cys Thr Asn Lys His Gly Leu Ser Asn Ser Ile Tyr Val Phe Val Arg
100 105 110

Asp Pro Ala Lys Leu Phe Leu Val Asp Arg Ser Leu Tyr Gly Lys Glu
115 120 125

Asp Asn Asp Thr Leu Val Arg Cys Pro Leu Thr Asp Pro Glu Val Thr
130 135 140

Asn Tyr Ser Leu Lys Gly Cys Gln Gly Lys Pro Leu Pro Lys Asp Leu
145 150 155 160

Arg Phe Ile Pro Asp Pro Lys Ala Gly Ile Met Ile Lys Ser Val Lys
165 170 175

Arg Ala Tyr His Arg Leu Cys Leu His Cys Ser Val Asp Gln Glu Gly

180 185 190

Lys Ser Val Leu Ser Glu Lys Phe Ile Leu Lys Val Arg Pro Ala Phe
195 200 205

Lys Ala Val Pro Val Val Ser Val Ser Lys Ala Ser Tyr Leu Leu Arg
210 215 220

Glu Gly Glu Glu Phe Thr Val Thr Cys Thr Ile Lys Asp Val Ser Ser
225 230 235 240

Ser Val Tyr Ser Thr Trp Lys Arg Glu Asn Ser Gln Thr Lys Leu Gln
245 250 255

Glu Lys Tyr Asn Ser Trp His His Gly Asp Phe Asn Tyr Glu Arg Gln
260 265 270

Ala Thr Leu Thr Ile Ser Ser Ala Arg Val Asn Asp Ser Gly Val Phe
275 280 285

Met Cys Tyr Ala Asn Asn Thr Phe Gly Ser Ala Asn Val Thr Thr Thr
290 295 300

Leu Glu Val Val Asp Lys Gly Phe Ile Asn Ile Phe Pro Met Ile Asn
305 310 315 320

Thr Thr Val Phe Val Asn Asp Gly Glu Asn Val Asp Leu Ile Val Glu
325 330 335

Tyr Glu Ala Phe Pro Lys Pro Glu His Gln Gln Trp Ile Tyr Met Asn
340 345 350

Arg Thr Phe Thr Asp Lys Trp Glu Asp Tyr Pro Lys Ser Glu Asn Glu
355 360 365

Ser Asn Ile Arg Tyr Val Ser Glu Leu His Leu Thr Arg Leu Lys Gly
370 375 380

Thr Glu Gly Gly Thr Tyr Thr Phe Leu Val Ser Asn Ser Asp Val Asn
385 390 395 400

Ala Ala Ile Ala Phe Asn Val Tyr Val Asn Thr Lys Pro Glu Ile Leu
405 410 415

Thr Tyr Asp Arg Leu Val Asn Gly Met Leu Gln Cys Val Ala Ala Gly
420 425 430

Phe Pro Glu Pro Thr Ile Asp Trp Tyr Phe Cys Pro Gly Thr Glu Gln
435 440 445

Arg Cys Ser Ala Ser Val Leu Pro Val Asp Val Gln Thr Leu Asn Ser
450 455 460

Ser Gly Pro Pro Phe Gly Lys Leu Val Val Gln Ser Ser Ile Asp Ser
465 470 475 480

Ser Ala Phe Lys His Asn Gly Thr Val Glu Cys Lys Ala Tyr Asn Asp
485 490 495

Val Gly Lys Thr Ser Ala Tyr Phe Asn Phe Ala Phe Lys Gly Asn Asn
500 505 510

Lys Glu Gln Ile His Pro His Thr Leu Phe Thr Pro Leu Leu Ile Gly
515 520 525

Phe Val Ile Val Ala Gly Met Met Cys Ile Ile Val Met Ile Leu Thr
530 535 540

Tyr Lys Tyr Leu Gln Lys Pro Met Tyr Glu Val Gln Trp Lys Val Val
545 550 555 560

Glu Glu Ile Asn Gly Asn Asn Tyr Val Tyr Ile Asp Pro Thr Gln Leu
565 570 575

Pro Tyr Asp His Lys Trp Glu Phe Pro Arg Asn Arg Leu Ser Phe Gly
580 585 590

Lys Thr Leu Gly Ala Gly Ala Phe Gly Lys Val Val Glu Ala Thr Ala
595 600 605

Tyr Gly Leu Ile Lys Ser Asp Ala Ala Met Thr Val Ala Val Lys Met
610 615 620

Leu Lys Pro Ser Ala His Leu Thr Glu Arg Glu Ala Leu Met Ser Glu
625 630 635 640

Leu Lys Val Leu Ser Tyr Leu Gly Asn His Met Asn Ile Val Asn Leu
645 650 655

Leu Gly Ala Cys Thr Ile Gly Gly Pro Thr Leu Val Ile Thr Glu Tyr
660 665 670

Cys Cys Tyr Gly Asp Leu Leu Asn Phe Leu Arg Arg Lys Arg Asp Ser
675 680 685

Phe Ile Cys Ser Lys Gln Glu Asp His Ala Glu Ala Ala Leu Tyr Lys
690 695 700

Asn Leu Leu His Ser Lys Glu Ser Ser Cys Ser Asp Ser Thr Asn Glu
705 710 715 720

Tyr Met Asp Met Lys Pro Gly Val Ser Tyr Val Val Pro Thr Lys Ala
725 730 735

Asp Lys Arg Arg Ser Val Arg Ile Gly Ser Tyr Ile Glu Arg Asp Val
740 745 750

Thr Pro Ala Ile Met Glu Asp Asp Glu Leu Ala Leu Asp Leu Glu Asp
755 760 765

Leu Leu Ser Phe Ser Tyr Gln Val Ala Lys Gly Met Ala Phe Leu Ala
770 775 780

Ser Lys Asn Cys Ile His Arg Asp Leu Ala Ala Arg Asn Ile Leu Leu
785 790 795 800

Thr His Gly Arg Ile Thr Lys Ile Cys Asp Phe Gly Leu Ala Arg Asp
805 810 815

Ile Lys Asn Asp Ser Asn Tyr Val Val Lys Gly Asn Ala Arg Leu Pro
820 825 830

Val Lys Trp Met Ala Pro Glu Ser Ile Phe Asn Cys Val Tyr Thr Phe
835 840 845

Glu Ser Asp Val Trp Ser Tyr Gly Ile Phe Leu Trp Glu Leu Phe Ser
850 855 860

Leu Gly Ser Ser Pro Tyr Pro Gly Met Pro Val Asp Ser Lys Phe Tyr
865 870 875 880

Lys Met Ile Lys Glu Gly Phe Arg Met Leu Ser Pro Glu His Ala Pro
885 890 895

Ala Glu Met Tyr Asp Ile Met Lys Thr Cys Trp Asp Ala Asp Pro Leu
900 905 910

Lys Arg Pro Thr Phe Lys Gln Ile Val Gln Leu Ile Glu Lys Gln Ile
915 920 925

Ser Glu Ser Thr Asn His Ile Tyr Ser Asn Leu Ala Asn Cys Ser Pro
930 935 940

Asn Arg Gln Lys Pro Val Val Asp His Ser Val Arg Ile Asn Ser Val
945 950 955 960

Gly Ser Thr Ala Ser Ser Ser Gln Pro Leu Leu Val His Asp Asp Val
965 970 975

<210> 15
<211> 25
<212> DNA
<213> artificial sequence

<220>
<223> RDC-1-specific PMO antisense oligonucleotide

<400> 15
gaagagatgc agatccatcg ttctg 25

<210> 16
<211> 25
<212> DNA
<213> artificial sequence

<220>
<223> IGFBP-2-specific PMO antisense oligonucleotide

<400> 16
ggcagcccac tctctcggca gcatg 25

<210> 17
<211> 25
<212> DNA
<213> artificial sequence

<220>
<223> FLJ14103-specific PMO antisense oligonucleotide

<400> 17
ggctccatct tgggctctgg gctcc 25

<210> 18
<211> 25
<212> DNA
<213> artificial sequence

<220>
<223> KIAA0367-specific PMO antisense oligonucleotide

<400> 18
gtcagtttac tcatgtcatc tattg 25

<210> 19
<211> 25
<212> DNA
<213> artificial sequence

<220>
<223> Neuritin-specific PMO antisense oligonucleotide

<400> 19
ttaactccca tcctacgttt agtca 25

<210> 20
<211> 22
<212> DNA
<213> artificial sequence

<220>
<223> INSR-specific PMO antisense oligonucleotide

<400> 20
gggtctcctc ggatcaggcg cg 22

<210> 21
<211> 23
<212> DNA
<213> artificial sequence

<220>
<223> KIT-specific PMO antisense oligonucleotide

<400> 21
cgcctctcat cgcggtagct gcg 23

<210> 22
<211> 22
<212> DNA
<213> artificial sequence

<220>
<223> IFACTOR-specific PMO antisense oligonucleotide

<400> 22
agcttcatgt tggaggtgtt cg 22

<210> 23
<211> 25
<212> DNA
<213> artificial sequence

<220>
<223> LMO2-specific PMO antisense oligonucleotide

<400> 23
gccgaggaca ttggggaggg aggcg 25

<210> 24
<211> 25
<212> DNA
<213> artificial sequence

<220>
<223> MFAP3-specific PMO antisense oligonucleotide

<400> 24
tgaataagca acaatgtagc ttcat 25

<210> 25
<211> 1946
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (274)..(1527)

<400> 25
agacactgcc cgctctccgg gactccgcgc cgctccccgt tgccttccag gactgagaaa 60

ggggaaaggg aagggtgcca cgtccgagca gccgccttga ctggggaagg gtctgaatcc 120

cacccttggc attgcttggt ggagactgag atacccgtgc tccgctcgcc tccttggttg 180

aagatttctc cttccctcac gtgatttgag ccccgttttt attttctgtg agccacgtcc 240

tcctcgagcg gggtcaatct ggcaaaagga gtg atg cgc ttc gcc tgg acc gtg 294
Met Arg Phe Ala Trp Thr Val
1 5

ctc ctg ctc ggg cct ttg cag ctc tgc gcg cta gtg cac tgc gcc cct 342
Leu Leu Leu Gly Pro Leu Gln Leu Cys Ala Leu Val His Cys Ala Pro
10 15 20

ccc gcc gcc ggc caa cag cag ccc ccg cgc gag ccg ccg gcg gct ccg 390
Pro Ala Ala Gly Gln Gln Gln Pro Pro Arg Glu Pro Pro Ala Ala Pro
25 30 35

ggc gcc tgg cgc cag cag atc caa tgg gag aac aac ggg cag gtg ttc 438
Gly Ala Trp Arg Gln Gln Ile Gln Trp Glu Asn Asn Gly Gln Val Phe
40 45 50 55

agc ttg ctg agc ctg ggc tca cag tac cag cct cag cgc cgc cgg gac 486
Ser Leu Leu Ser Leu Gly Ser Gln Tyr Gln Pro Gln Arg Arg Arg Asp
60 65 70

ccg ggc gcc gcc gtc cct ggt gca gcc aac gcc tcc gcc cag cag ccc 534
Pro Gly Ala Ala Val Pro Gly Ala Ala Asn Ala Ser Ala Gln Gln Pro
75 80 85

cgc act ccg atc ctg ctg atc cgc gac aac cgc acc gcc gcg gcg cga 582
Arg Thr Pro Ile Leu Leu Ile Arg Asp Asn Arg Thr Ala Ala Ala Arg
90 95 100

acg cgg acg gcc ggc tca tct gga gtc acc gct ggc cgc ccc agg ccc 630
Thr Arg Thr Ala Gly Ser Ser Gly Val Thr Ala Gly Arg Pro Arg Pro
105 110 115

acc gcc cgt cac tgg ttc caa gct ggc tac tcg aca tct aga gcc cgc 678
Thr Ala Arg His Trp Phe Gln Ala Gly Tyr Ser Thr Ser Arg Ala Arg
120 125 130 135

gaa gct ggc gcc tcg cgc gcg gag aac cag aca gcg ccg gga gaa gtt 726
Glu Ala Gly Ala Ser Arg Ala Glu Asn Gln Thr Ala Pro Gly Glu Val
140 145 150

```
cct gcg ctc agt aac ctg cgg ccg ccc agc cgc gtg gac ggc atg gtg      774
Pro Ala Leu Ser Asn Leu Arg Pro Pro Ser Arg Val Asp Gly Met Val
            155                 160                 165

ggc gac gac cct tac aac ccc tac aag tac tct gac gac aac cct tat      822
Gly Asp Asp Pro Tyr Asn Pro Tyr Lys Tyr Ser Asp Asp Asn Pro Tyr
        170                 175                 180

tac aac tac tac gat act tat gaa agg ccc aga cct ggg ggc agg tac      870
Tyr Asn Tyr Tyr Asp Thr Tyr Glu Arg Pro Arg Pro Gly Gly Arg Tyr
    185                 190                 195

cgg ccc gga tac ggc act ggc tac ttc cag tac ggt ctc cca gac ctg      918
Arg Pro Gly Tyr Gly Thr Gly Tyr Phe Gln Tyr Gly Leu Pro Asp Leu
200                 205                 210                 215

gtg gcc gac ccc tac tac atc cag gcg tcc acg tac gtg cag aag atg      966
Val Ala Asp Pro Tyr Tyr Ile Gln Ala Ser Thr Tyr Val Gln Lys Met
                220                 225                 230

tcc atg tac aac ctg aga tgc gcg gcg gag gaa aac tgt ctg gcc agt     1014
Ser Met Tyr Asn Leu Arg Cys Ala Ala Glu Glu Asn Cys Leu Ala Ser
            235                 240                 245

aca gca tac agg gca gat gtc aga gat tat gat cac agg gtg ctg ctc     1062
Thr Ala Tyr Arg Ala Asp Val Arg Asp Tyr Asp His Arg Val Leu Leu
        250                 255                 260

aga ttt ccc caa aga gtg aaa aac caa ggg aca tca gat ttc tta ccc     1110
Arg Phe Pro Gln Arg Val Lys Asn Gln Gly Thr Ser Asp Phe Leu Pro
    265                 270                 275

agc cga cca aga tat tcc tgg gaa tgg cac agt tgt cat caa cat tac     1158
Ser Arg Pro Arg Tyr Ser Trp Glu Trp His Ser Cys His Gln His Tyr
280                 285                 290                 295

cac agt atg gat gag ttt agc cac tat gac ctg ctt gat gcc aac acc     1206
His Ser Met Asp Glu Phe Ser His Tyr Asp Leu Leu Asp Ala Asn Thr
                300                 305                 310

cag agg aga gtg gct gaa ggc cac aaa gca agt ttc tgt ctt gaa gac     1254
Gln Arg Arg Val Ala Glu Gly His Lys Ala Ser Phe Cys Leu Glu Asp
            315                 320                 325

aca tcc tgt gac tat ggc tac cac agg cga ttt gca tgt act gca cac     1302
Thr Ser Cys Asp Tyr Gly Tyr His Arg Arg Phe Ala Cys Thr Ala His
        330                 335                 340

aca cag gga ttg agt cct ggc tgt tat gat acc tat ggt gca gac ata     1350
Thr Gln Gly Leu Ser Pro Gly Cys Tyr Asp Thr Tyr Gly Ala Asp Ile
    345                 350                 355

gac tgc cag tgg att gat att aca gat gta aaa cct gga aac tat atc     1398
Asp Cys Gln Trp Ile Asp Ile Thr Asp Val Lys Pro Gly Asn Tyr Ile
360                 365                 370                 375

cta aag gtc agt gta aac ccc agc tac ctg gtt cct gaa tct gac tat     1446
Leu Lys Val Ser Val Asn Pro Ser Tyr Leu Val Pro Glu Ser Asp Tyr
                380                 385                 390

acc aac aat gtt gtg cgc tgt gac att cgc tac aca gga cat cat gcg     1494
Thr Asn Asn Val Val Arg Cys Asp Ile Arg Tyr Thr Gly His His Ala
            395                 400                 405
```

tat gcc tca ggc tgc aca att tca ccg tat tag aaggcaaagc aaaactccca 1547
Tyr Ala Ser Gly Cys Thr Ile Ser Pro Tyr
410 415

atggataaat cagtgcctgg tgttctgaag tgggaaaaaa tagactaact tcagtaggat 1607

ttatgtattt tgaaaaagag aacagaaaac aacaaaagaa tttttgtttg gactgttttc 1667

aataacaaag cacataactg gattttgaac gcttaagtca tcattacttg ggaaattttt 1727

aatgtttatt atttacatca ctttgtgaat taacacagtg tttcaattct gtaattacat 1787

atttgactct ttcaaagaaa tccaaatttc tcatgttcct tttgaaattg tagtgcaaaa 1847

tggtcagtat tatctaaatg aatgagccaa aatgactttg aactgaaact tttctaaagt 1907

gctggaactt tagtgaaaca taataataat gggtttata 1946

<210> 26
<211> 417
<212> PRT
<213> Homo sapiens

<400> 26

Met Arg Phe Ala Trp Thr Val Leu Leu Leu Gly Pro Leu Gln Leu Cys
1 5 10 15

Ala Leu Val His Cys Ala Pro Pro Ala Ala Gly Gln Gln Gln Pro Pro
20 25 30

Arg Glu Pro Pro Ala Ala Pro Gly Ala Trp Arg Gln Gln Ile Gln Trp
35 40 45

Glu Asn Asn Gly Gln Val Phe Ser Leu Leu Ser Leu Gly Ser Gln Tyr
50 55 60

Gln Pro Gln Arg Arg Arg Asp Pro Gly Ala Ala Val Pro Gly Ala Ala
65 70 75 80

Asn Ala Ser Ala Gln Gln Pro Arg Thr Pro Ile Leu Leu Ile Arg Asp
85 90 95

Asn Arg Thr Ala Ala Ala Arg Thr Arg Thr Ala Gly Ser Ser Gly Val
100 105 110

Thr Ala Gly Arg Pro Arg Pro Thr Ala Arg His Trp Phe Gln Ala Gly
115 120 125

Tyr Ser Thr Ser Arg Ala Arg Glu Ala Gly Ala Ser Arg Ala Glu Asn
130 135 140

Gln Thr Ala Pro Gly Glu Val Pro Ala Leu Ser Asn Leu Arg Pro Pro
145 150 155 160

Ser Arg Val Asp Gly Met Val Gly Asp Asp Pro Tyr Asn Pro Tyr Lys
165 170 175

Tyr Ser Asp Asp Asn Pro Tyr Tyr Asn Tyr Tyr Asp Thr Tyr Glu Arg
180 185 190

Pro Arg Pro Gly Gly Arg Tyr Arg Pro Gly Tyr Gly Thr Gly Tyr Phe
195 200 205

Gln Tyr Gly Leu Pro Asp Leu Val Ala Asp Pro Tyr Tyr Ile Gln Ala
210 215 220

Ser Thr Tyr Val Gln Lys Met Ser Met Tyr Asn Leu Arg Cys Ala Ala
225 230 235 240

Glu Glu Asn Cys Leu Ala Ser Thr Ala Tyr Arg Ala Asp Val Arg Asp
245 250 255

Tyr Asp His Arg Val Leu Leu Arg Phe Pro Gln Arg Val Lys Asn Gln
260 265 270

Gly Thr Ser Asp Phe Leu Pro Ser Arg Pro Arg Tyr Ser Trp Glu Trp
275 280 285

His Ser Cys His Gln His Tyr His Ser Met Asp Glu Phe Ser His Tyr
290 295 300

Asp Leu Leu Asp Ala Asn Thr Gln Arg Arg Val Ala Glu Gly His Lys
305 310 315 320

Ala Ser Phe Cys Leu Glu Asp Thr Ser Cys Asp Tyr Gly Tyr His Arg
325 330 335

Arg Phe Ala Cys Thr Ala His Thr Gln Gly Leu Ser Pro Gly Cys Tyr
340 345 350

Asp Thr Tyr Gly Ala Asp Ile Asp Cys Gln Trp Ile Asp Ile Thr Asp
355 360 365

Val Lys Pro Gly Asn Tyr Ile Leu Lys Val Ser Val Asn Pro Ser Tyr
370 375 380

Leu Val Pro Glu Ser Asp Tyr Thr Asn Asn Val Val Arg Cys Asp Ile
385 390 395 400

```
Arg Tyr Thr Gly His His Ala Tyr Ala Ser Gly Cys Thr Ile Ser Pro
                405                 410                 415

Tyr
```

<210> 27
<211> 2389
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (73)..(1146)

<400> 27

```
gggaaggcga gcagtgccaa tctacagcga agaaagtctc gtttggtaaa agcgagaggg      60

gaaagcctga gc atg cag agt gtg cag agc acg agc ttt tgt ctc cga aag     111
              Met Gln Ser Val Gln Ser Thr Ser Phe Cys Leu Arg Lys
              1               5                   10

cag tgc ctt tgc ctg acc ttc ctg ctt ctc cat ctc ctg gga cag gtc     159
Gln Cys Leu Cys Leu Thr Phe Leu Leu Leu His Leu Leu Gly Gln Val
    15                  20                  25

gct gcg act cag cgc tgc cct ccc cag tgc ccg ggc cgg tgc cct gcg     207
Ala Ala Thr Gln Arg Cys Pro Pro Gln Cys Pro Gly Arg Cys Pro Ala
30                  35                  40                  45

acg ccg ccg acc tgc gcc ccc ggg gtg cgc gcg gtg ctg gac ggc tgc     255
Thr Pro Pro Thr Cys Ala Pro Gly Val Arg Ala Val Leu Asp Gly Cys
                50                  55                  60

tca tgc tgt ctg gtg tgt gcc cgc cag cgt ggc gag agc tgc tca gat     303
Ser Cys Cys Leu Val Cys Ala Arg Gln Arg Gly Glu Ser Cys Ser Asp
            65                  70                  75

ctg gag cca tgc gac gag agc agt ggc ctc tac tgt gat cgc agc gcg     351
Leu Glu Pro Cys Asp Glu Ser Ser Gly Leu Tyr Cys Asp Arg Ser Ala
        80                  85                  90

gac ccc agc aac cag act ggc atc tgc acg gcg gta gag gga gat aac     399
Asp Pro Ser Asn Gln Thr Gly Ile Cys Thr Ala Val Glu Gly Asp Asn
    95                  100                 105

tgt gtg ttc gat ggg gtc atc tac cgc agt gga gag aaa ttt cag cca     447
Cys Val Phe Asp Gly Val Ile Tyr Arg Ser Gly Glu Lys Phe Gln Pro
110                 115                 120                 125

agc tgc aaa ttc cag tgc acc tgc aga gat ggg cag att ggc tgt gtg     495
Ser Cys Lys Phe Gln Cys Thr Cys Arg Asp Gly Gln Ile Gly Cys Val
                130                 135                 140

ccc cgc tgt cag ctg gat gtg cta ctg cct gag cct aac tgc cca gct     543
Pro Arg Cys Gln Leu Asp Val Leu Leu Pro Glu Pro Asn Cys Pro Ala
            145                 150                 155

cca aga aaa gtt gag gtg cct gga gag tgc tgt gaa aag tgg atc tgt     591
```

```
Pro Arg Lys Val Glu Val Pro Gly Glu Cys Cys Glu Lys Trp Ile Cys
        160                 165                 170

ggc cca gat gag gag gat tca ctg gga ggc ctt acc ctt gca gct tac      639
Gly Pro Asp Glu Glu Asp Ser Leu Gly Gly Leu Thr Leu Ala Ala Tyr
    175                 180                 185

agg cca gaa gcc acc cta gga gta gaa gtc tct gac tca agt gtc aac      687
Arg Pro Glu Ala Thr Leu Gly Val Glu Val Ser Asp Ser Ser Val Asn
190                 195                 200                 205

tgc att gaa cag acc aca gag tgg aca gca tgc tcc aag agc tgt ggt      735
Cys Ile Glu Gln Thr Thr Glu Trp Thr Ala Cys Ser Lys Ser Cys Gly
                210                 215                 220

atg ggg ttc tcc acc cgg gtc acc aat agg aac cgt caa tgt gag atg      783
Met Gly Phe Ser Thr Arg Val Thr Asn Arg Asn Arg Gln Cys Glu Met
            225                 230                 235

ctg aaa cag act cgg ctc tgc atg gtg cgg ccc tgt gaa caa gag cca      831
Leu Lys Gln Thr Arg Leu Cys Met Val Arg Pro Cys Glu Gln Glu Pro
        240                 245                 250

gag cag cca aca gat aag aaa gga aaa aag tgt ctc cgc acc aag aag      879
Glu Gln Pro Thr Asp Lys Lys Gly Lys Lys Cys Leu Arg Thr Lys Lys
    255                 260                 265

tca ctc aaa gcc atc cac ctg cag ttc aag aac tgc acc agc ctg cac      927
Ser Leu Lys Ala Ile His Leu Gln Phe Lys Asn Cys Thr Ser Leu His
270                 275                 280                 285

acc tac aag ccc agg ttc tgt ggg gtc tgc agt gat ggc cgc tgc tgc      975
Thr Tyr Lys Pro Arg Phe Cys Gly Val Cys Ser Asp Gly Arg Cys Cys
                290                 295                 300

act ccc cac aat acc aaa acc atc cag gca gag ttt cag tgc tcc cca     1023
Thr Pro His Asn Thr Lys Thr Ile Gln Ala Glu Phe Gln Cys Ser Pro
            305                 310                 315

ggg caa ata gtc aag aag cca gtg atg gtc att ggg acc tgc acc tgt     1071
Gly Gln Ile Val Lys Lys Pro Val Met Val Ile Gly Thr Cys Thr Cys
        320                 325                 330

cac acc aac tgt cct aag aac aat gag gcc ttc ctc cag gag ctg gag     1119
His Thr Asn Cys Pro Lys Asn Asn Glu Ala Phe Leu Gln Glu Leu Glu
    335                 340                 345

ctg aag act acc aga ggg aaa atg taa cctatcactc aagaagcaca           1166
Leu Lys Thr Thr Arg Gly Lys Met
350                 355

cctacagagc acctgtagct gctgcgccac ccaccatcaa aggaatataa gaaaagtaat   1226

gaagaatcac gatttcatcc ttgaatccta tgtattttcc taatgtgatc atatgaggac   1286

ctttcatatc tgtcttttat ttaacaaaaa atgtaattaa ctgtaaactt ggaatcaagg   1346

taagctcagg atatggctta ggaatgactt actttcctgt ggttttatta caaatgcaaa   1406

tttctataaa tttaagaaaa caagtatata atttactttg tagactgttt cacattgcac   1466

tcatcatatt ttgttgtgca ctagtgcaat tccaagaaaa tatcactgta atgagtcagt   1526
```

gaagtctaga atcatactta acatttcatt gtacaagtat tacaaccata tattgaggtt 1586

cattgggaag attctctatt ggctcccttt ttgggtaaac cagctctgaa cttccaagct 1646

ccaaatccaa ggaaacatgc agctcttcaa catgacatcc agagatgact attacttttc 1706

tgtttagttt tacactagga aacgtgttgt atctacagta atgaaatgtt tactaagtgg 1766

actggtgtca taaactttct ccatttaaga cacattgact cctttccaat agaaagaaac 1826

taaacagaaa actcccaata caaagatgac tggtccctca tagccctcag acatttatat 1886

attggaagct gctgaggccc ccaagttttt taattaagca gaaacagcat attagcaggg 1946

attctctcat ctaactgatg agtaaactga ggcccaaagc acttgcttac atcctctgat 2006

agctgtttca aatgtgcatt ttgtggaatt ttgagaaaaa tagagcaaaa tcaacatgac 2066

tggtggtgag agaccacaca ttttatgaga gtttggaatt attgtagaca tgcccaaaac 2126

ttatccttgg gccataatta tgaaaactca tgatcaagat atatgtgtat acatacatgt 2186

atctggtttg tcaggctaca aggtaggctg caaaattaaa tctagacatt cttttaatgc 2246

caccacacgt gttccgcttc tctcttttaa agtatttata aaaatataaa ttgtacattt 2306

tgtaaaatat tatgtttgat ttctctactt gtcatatcac taaataaaca cgattttatt 2366

gctgaaaaaa aaaaaaaaaa aaa 2389

<210> 28
<211> 357
<212> PRT
<213> Homo sapiens

<400> 28

Met Gln Ser Val Gln Ser Thr Ser Phe Cys Leu Arg Lys Gln Cys Leu
1 5 10 15

Cys Leu Thr Phe Leu Leu Leu His Leu Leu Gly Gln Val Ala Ala Thr
20 25 30

Gln Arg Cys Pro Pro Gln Cys Pro Gly Arg Cys Pro Ala Thr Pro Pro
35 40 45

Thr Cys Ala Pro Gly Val Arg Ala Val Leu Asp Gly Cys Ser Cys Cys
50 55 60

Leu Val Cys Ala Arg Gln Arg Gly Glu Ser Cys Ser Asp Leu Glu Pro
65 70 75 80

Cys Asp Glu Ser Ser Gly Leu Tyr Cys Asp Arg Ser Ala Asp Pro Ser
85 90 95

Asn Gln Thr Gly Ile Cys Thr Ala Val Glu Gly Asp Asn Cys Val Phe

100 105 110

Asp Gly Val Ile Tyr Arg Ser Gly Glu Lys Phe Gln Pro Ser Cys Lys
115 120 125

Phe Gln Cys Thr Cys Arg Asp Gly Gln Ile Gly Cys Val Pro Arg Cys
130 135 140

Gln Leu Asp Val Leu Leu Pro Glu Pro Asn Cys Pro Ala Pro Arg Lys
145 150 155 160

Val Glu Val Pro Gly Glu Cys Cys Glu Lys Trp Ile Cys Gly Pro Asp
165 170 175

Glu Glu Asp Ser Leu Gly Gly Leu Thr Leu Ala Ala Tyr Arg Pro Glu
180 185 190

Ala Thr Leu Gly Val Glu Val Ser Asp Ser Ser Val Asn Cys Ile Glu
195 200 205

Gln Thr Thr Glu Trp Thr Ala Cys Ser Lys Ser Cys Gly Met Gly Phe
210 215 220

Ser Thr Arg Val Thr Asn Arg Asn Arg Gln Cys Glu Met Leu Lys Gln
225 230 235 240

Thr Arg Leu Cys Met Val Arg Pro Cys Glu Gln Glu Pro Glu Gln Pro
245 250 255

Thr Asp Lys Lys Gly Lys Lys Cys Leu Arg Thr Lys Lys Ser Leu Lys
260 265 270

Ala Ile His Leu Gln Phe Lys Asn Cys Thr Ser Leu His Thr Tyr Lys
275 280 285

Pro Arg Phe Cys Gly Val Cys Ser Asp Gly Arg Cys Cys Thr Pro His
290 295 300

Asn Thr Lys Thr Ile Gln Ala Glu Phe Gln Cys Ser Pro Gly Gln Ile
305 310 315 320

Val Lys Lys Pro Val Met Val Ile Gly Thr Cys Thr Cys His Thr Asn
325 330 335

Cys Pro Lys Asn Asn Glu Ala Phe Leu Gln Glu Leu Glu Leu Lys Thr
340 345 350

Thr Arg Gly Lys Met
355

<210> 29
<211> 1518
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (22)..(1503)

<400> 29
aaccaccatt ttgcaaggac c atg agg cca ctg tgc gtg aca tgc tgg tgg 51
Met Arg Pro Leu Cys Val Thr Cys Trp Trp
1 5 10

ctc gga ctg ctg gct gcc atg gga gct gtt gca ggc cag gag gac ggt 99
Leu Gly Leu Leu Ala Ala Met Gly Ala Val Ala Gly Gln Glu Asp Gly
15 20 25

ttt gag ggc act gag gag ggc tcg cca aga gag ttc att tac cta aac 147
Phe Glu Gly Thr Glu Glu Gly Ser Pro Arg Glu Phe Ile Tyr Leu Asn
30 35 40

agg tac aag cgg gcg ggc gag tcc cag gac aag tgc acc tac acc ttc 195
Arg Tyr Lys Arg Ala Gly Glu Ser Gln Asp Lys Cys Thr Tyr Thr Phe
45 50 55

att gtg ccc cag cag cgg gtc acg ggt gcc atc tgc gtc aac tcc aag 243
Ile Val Pro Gln Gln Arg Val Thr Gly Ala Ile Cys Val Asn Ser Lys
60 65 70

gag cct gag gtg ctt ctg gag aac cga gtg cat aag cag gag cta gag 291
Glu Pro Glu Val Leu Leu Glu Asn Arg Val His Lys Gln Glu Leu Glu
75 80 85 90

ctg ctc aac aat gag ctg ctc aag cag aag cgg cag atc gag aca ctg 339
Leu Leu Asn Asn Glu Leu Leu Lys Gln Lys Arg Gln Ile Glu Thr Leu
95 100 105

cag cag ctg gtg gag gtg gac ggc ggc att gtg agc gag gtg aag ctg 387
Gln Gln Leu Val Glu Val Asp Gly Gly Ile Val Ser Glu Val Lys Leu
110 115 120

ctg cgc aag gag agc cgc aac atg aac tcg cgg gtc acg cag ctc tac 435
Leu Arg Lys Glu Ser Arg Asn Met Asn Ser Arg Val Thr Gln Leu Tyr
125 130 135

atg cag ctc ctg cac gag atc atc cgc aag cgg gac aac gcg ttg gag 483
Met Gln Leu Leu His Glu Ile Ile Arg Lys Arg Asp Asn Ala Leu Glu
140 145 150

ctc tcc cag ctg gag aac agg atc ctg aac cag aca gcc gac atg ctg 531
Leu Ser Gln Leu Glu Asn Arg Ile Leu Asn Gln Thr Ala Asp Met Leu
155 160 165 170

cag ctg gcc agc aag tac aag gac ctg gag cac aag tac cag cac ctg 579
Gln Leu Ala Ser Lys Tyr Lys Asp Leu Glu His Lys Tyr Gln His Leu
175 180 185

```
gcc aca ctg gcc cac aac caa tca gag atc atc gcg cag ctt gag gag      627
Ala Thr Leu Ala His Asn Gln Ser Glu Ile Ile Ala Gln Leu Glu Glu
            190                 195                 200

cac tgc cag agg gtg ccc tcg gcc agg ccc gtc ccc cag cca ccc ccc      675
His Cys Gln Arg Val Pro Ser Ala Arg Pro Val Pro Gln Pro Pro Pro
        205                 210                 215

gct gcc ccg ccc cgg gtc tac caa cca ccc acc tac aac cgc atc atc      723
Ala Ala Pro Pro Arg Val Tyr Gln Pro Pro Thr Tyr Asn Arg Ile Ile
    220                 225                 230

aac cag atc tct acc aac gag atc cag agt gac cag aac ctg aag gtg      771
Asn Gln Ile Ser Thr Asn Glu Ile Gln Ser Asp Gln Asn Leu Lys Val
235                 240                 245                 250

ctg cca ccc cct ctg ccc act atg ccc act ctc acc agc ctc cca tct      819
Leu Pro Pro Pro Leu Pro Thr Met Pro Thr Leu Thr Ser Leu Pro Ser
                255                 260                 265

tcc acc gac aag ccg tcg ggc cca tgg aga gac tgc ctg cag gcc ctg      867
Ser Thr Asp Lys Pro Ser Gly Pro Trp Arg Asp Cys Leu Gln Ala Leu
            270                 275                 280

gag gat ggc cac gac acc agc tcc atc tac ctg gtg aag ccg gag aac      915
Glu Asp Gly His Asp Thr Ser Ser Ile Tyr Leu Val Lys Pro Glu Asn
        285                 290                 295

acc aac cgc ctc atg cag gtg tgg tgc gac cag aga cac gac ccc ggg      963
Thr Asn Arg Leu Met Gln Val Trp Cys Asp Gln Arg His Asp Pro Gly
    300                 305                 310

ggc tgg acc gtc atc cag aga cgc ctg gat ggc tct gtt aac ttc ttc     1011
Gly Trp Thr Val Ile Gln Arg Arg Leu Asp Gly Ser Val Asn Phe Phe
315                 320                 325                 330

agg aac tgg gag acg tac aag caa ggg ttt ggg aac att gat ggc gaa     1059
Arg Asn Trp Glu Thr Tyr Lys Gln Gly Phe Gly Asn Ile Asp Gly Glu
                335                 340                 345

tac tgg ctg ggc ctg gag aac att tac tgg ctg acg aac caa ggc aac     1107
Tyr Trp Leu Gly Leu Glu Asn Ile Tyr Trp Leu Thr Asn Gln Gly Asn
            350                 355                 360

tac aaa ctc ctg gtg acc atg gag gac tgg tcc ggc cgc aaa gtc ttt     1155
Tyr Lys Leu Leu Val Thr Met Glu Asp Trp Ser Gly Arg Lys Val Phe
        365                 370                 375

gca gaa tac gcc agt ttc cgc ctg gaa cct gag agc gag tat tat aag     1203
Ala Glu Tyr Ala Ser Phe Arg Leu Glu Pro Glu Ser Glu Tyr Tyr Lys
    380                 385                 390

ctg cgg ctg ggg cgc tac cat ggc aat gcg ggt gac tcc ttt aca tgg     1251
Leu Arg Leu Gly Arg Tyr His Gly Asn Ala Gly Asp Ser Phe Thr Trp
395                 400                 405                 410

cac aac ggc aag cag ttc acc acc ctg gac aga gat cat gat gtc tac     1299
His Asn Gly Lys Gln Phe Thr Thr Leu Asp Arg Asp His Asp Val Tyr
                415                 420                 425

aca gga aac tgt gcc cac tac cag aag gga ggc tgg tgg tat aac gcc     1347
Thr Gly Asn Cys Ala His Tyr Gln Lys Gly Gly Trp Trp Tyr Asn Ala
            430                 435                 440
```

```
tgt gcc cac tcc aac ctc aac ggg gtc tgg tac cgc ggg ggc cat tac      1395
Cys Ala His Ser Asn Leu Asn Gly Val Trp Tyr Arg Gly Gly His Tyr
        445                 450                 455

cgg agc cgc tac cag gac gga gtc tac tgg gct gag ttc cga gga ggc      1443
Arg Ser Arg Tyr Gln Asp Gly Val Tyr Trp Ala Glu Phe Arg Gly Gly
    460                 465                 470

tct tac tca ctc aag aaa gtg gtg atg atg atc cga ccg aac ccc aac      1491
Ser Tyr Ser Leu Lys Lys Val Val Met Met Ile Arg Pro Asn Pro Asn
475                 480                 485                 490

acc ttc cac taa gccagctccc cctcc                                    1518
Thr Phe His
```

<210> 30
<211> 493
<212> PRT
<213> Homo sapiens

<400> 30

```
Met Arg Pro Leu Cys Val Thr Cys Trp Trp Leu Gly Leu Leu Ala Ala
1               5                   10                  15

Met Gly Ala Val Ala Gly Gln Glu Asp Gly Phe Glu Gly Thr Glu Glu
            20                  25                  30

Gly Ser Pro Arg Glu Phe Ile Tyr Leu Asn Arg Tyr Lys Arg Ala Gly
        35                  40                  45

Glu Ser Gln Asp Lys Cys Thr Tyr Thr Phe Ile Val Pro Gln Gln Arg
    50                  55                  60

Val Thr Gly Ala Ile Cys Val Asn Ser Lys Glu Pro Glu Val Leu Leu
65                  70                  75                  80

Glu Asn Arg Val His Lys Gln Glu Leu Glu Leu Leu Asn Asn Glu Leu
                85                  90                  95

Leu Lys Gln Lys Arg Gln Ile Glu Thr Leu Gln Gln Leu Val Glu Val
            100                 105                 110

Asp Gly Gly Ile Val Ser Glu Val Lys Leu Leu Arg Lys Glu Ser Arg
        115                 120                 125

Asn Met Asn Ser Arg Val Thr Gln Leu Tyr Met Gln Leu Leu His Glu
    130                 135                 140

Ile Ile Arg Lys Arg Asp Asn Ala Leu Glu Leu Ser Gln Leu Glu Asn
145                 150                 155                 160
```

Arg Ile Leu Asn Gln Thr Ala Asp Met Leu Gln Leu Ala Ser Lys Tyr
165 170 175

Lys Asp Leu Glu His Lys Tyr Gln His Leu Ala Thr Leu Ala His Asn
180 185 190

Gln Ser Glu Ile Ile Ala Gln Leu Glu Glu His Cys Gln Arg Val Pro
195 200 205

Ser Ala Arg Pro Val Pro Gln Pro Pro Pro Ala Ala Pro Pro Arg Val
210 215 220

Tyr Gln Pro Pro Thr Tyr Asn Arg Ile Ile Asn Gln Ile Ser Thr Asn
225 230 235 240

Glu Ile Gln Ser Asp Gln Asn Leu Lys Val Leu Pro Pro Pro Leu Pro
245 250 255

Thr Met Pro Thr Leu Thr Ser Leu Pro Ser Ser Thr Asp Lys Pro Ser
260 265 270

Gly Pro Trp Arg Asp Cys Leu Gln Ala Leu Glu Asp Gly His Asp Thr
275 280 285

Ser Ser Ile Tyr Leu Val Lys Pro Glu Asn Thr Asn Arg Leu Met Gln
290 295 300

Val Trp Cys Asp Gln Arg His Asp Pro Gly Gly Trp Thr Val Ile Gln
305 310 315 320

Arg Arg Leu Asp Gly Ser Val Asn Phe Phe Arg Asn Trp Glu Thr Tyr
325 330 335

Lys Gln Gly Phe Gly Asn Ile Asp Gly Glu Tyr Trp Leu Gly Leu Glu
340 345 350

Asn Ile Tyr Trp Leu Thr Asn Gln Gly Asn Tyr Lys Leu Leu Val Thr
355 360 365

Met Glu Asp Trp Ser Gly Arg Lys Val Phe Ala Glu Tyr Ala Ser Phe
370 375 380

Arg Leu Glu Pro Glu Ser Glu Tyr Tyr Lys Leu Arg Leu Gly Arg Tyr
385 390 395 400

His Gly Asn Ala Gly Asp Ser Phe Thr Trp His Asn Gly Lys Gln Phe

405 410 415

Thr Thr Leu Asp Arg Asp His Asp Val Tyr Thr Gly Asn Cys Ala His
420 425 430

Tyr Gln Lys Gly Gly Trp Trp Tyr Asn Ala Cys Ala His Ser Asn Leu
435 440 445

Asn Gly Val Trp Tyr Arg Gly Gly His Tyr Arg Ser Arg Tyr Gln Asp
450 455 460

Gly Val Tyr Trp Ala Glu Phe Arg Gly Gly Ser Tyr Ser Leu Lys Lys
465 470 475 480

Val Val Met Met Ile Arg Pro Asn Pro Asn Thr Phe His
485 490

<210> 31
<211> 25
<212> DNA
<213> artificial sequence

<220>
<223> LOX-specific PMO antisense oligonucleotide

<400> 31
ggagcacggt ccaggcgaag cgcat 25

<210> 32
<211> 25
<212> DNA
<213> artificial sequence

<220>
<223> NOV-specific PMO antisense oligonucleotide

<400> 32
agctcgtgct ctgcacactc tgcat 25

<210> 33
<211> 25
<212> DNA
<213> artificial sequence

<220>
<223> ANGPTL2-specific PMO antisense oligonucleotide

<400> 33
agcatgtcac gcacagtggc ctcat 25